약학박사학위논문

Optimization of analytical method for the quality control of ginseng preparations and comparative analysis of ginsenosides of wild Panax species using LC-ELSD and LC-Q-TOF-MS

2015년8월

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이 논문을 약학박사학위논문으로 제출함

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Abstract

Ginseng, the root of the *Panax* genus plant has been used as an herbal medicine in Asia for over two thousand years for its various health benefits including (but not limited to)antioxidant, anti-carcinogenic, anti-inflammatory, antihypertensive andanti-diabetic effects. The pharmacologically active compounds behind the claims of ginseng's efficacy are ginsenosides. More than 300 different ginsenosides have been identified from several *Panax* plants. Ginsenoside is triterpene glycoside with four rings and several sugar moieties; they are found exclusively in ginseng plants and are in higher concentration in their roots.

Qualitative and quantitative techniques for the analysis of ginsenosides are in demand to ensure quality control of ginseng. Korean Pharmacopeia (KP) and Japanese Pharmacopeia (JP) describe ginseng as it contains not less than 0.10% of ginsenoside Rg1 and not less than 0.20% of

ginsenoside Rb1. Chinese Pharmacopeia (CP) describes that ginseng should contains not less than 0.30% of Rg1+Re and not less than 0.20% of Rb1.This kind of standard is appropriate for the quality control of white ginseng and its powder. However, that most ginsenoside lose a part of its sugar moiety and transformed to less polar ginsenoside especially under the heat, in acidic condition, or by fermentation. Heating process converts the naturally occurring ginsenoside Rg1 and Rb1 into artifact ginsenosides such as gisenosides Rg3, Rg5, Rh1, and Rh2. Therefore, gisenoside Rg1 and Rb1 which have been used as quality markers of ginseng, do not express the quality of some ginseng products. Consequently, the official method for the quality control of ginseng is not applicable for the processed ginseng.

The aim of this study is to develop a new simple analytical method which can be applied to all kinds of ginseng products including processed ginseng. To achieve this goal, sample preparation method and analytical condition were optimized using design of experiment (DOE) technique. The optimal conditions for the extraction of Rg1 and Rb1, which are the major compounds for White ginseng and Red ginseng were found at 60% aqueous methanol. However, 80% aqueous methanol was better for the efficient extraction of ginsenoside Rk1 and Rg5. The result indicates that the less polar solvent system might be a better choice for the efficient extraction of a ginseng preparation containing a large amount of ginsenoside Rk1 and Rg5.In general, 70% aqueous methanol was superior to 60% or 80% methanol for the extraction of polar and less polar ginsenosides together. The coefficient of correlation (R^2) was higher than 0.994 for all calibration curves. The method sensitivity was measured in terms of the limit of detection (LOD), which ranged from 1−7 ng/ml. The limit of quantification (LOQ) was found 2−23 ng/ml.

Ginsenoside Rb1 and Rg1 are described in Pharmacopeia as the marker compounds for the quality control of ginseng. However, they are not adequate marker for the certain ginseng products as they are unstable and easily convert to less polar ginsenosides. To solve this problem, group quantitation of ginsenoside was applied. All kinds of ginsenosides were categorized into three groups as follows. Group A: protopanaxatriol (PPT) group includes Rg1 and Re; Group B: protopanaxadiol (PPD) group includes Rb1, Rb2, Rc and Rd; Group C: less polar ginsenosides group includes Rg3, Rk1 and Rg5. The contents of ginsenosides in Group A, B and C were expressed as the contents of ginsenosides Rg1, Rb1, and Rg3, respectively. The group quantitation method was successfully applied for the quantitative analysis of White ginseng and heat-treated ginseng products. The contents of ginsenoside Rg1 and Rb1 in White ginseng were 0.37 mg/g and 1.41mg/g, respectively. The content of PPT group and PPD group in the same sample were 1.23 mg/g and 3.91mg/g, respectively which gives better information about the total content of ginsenosides and, consequently, quality of ginseng. Sum of all ginsenosides was 5.14 mg/g.

Due to the steaming process the contents of ginsenoside Rg1 (0.08 mg/g) and Rb1 (0.78 mg/g) in processed ginseng were degraded, which does not meet the requirements of the Pharmacopoeia. The content of group analysis in the same sample was as follows: 0.38 mg/g for PPT group, 3.16 mg/g for PPD group and 1.90 mg/g for less polar gisenosides group. Sum of all ginsenosides was 5.44 mg/g.

Group analysis method is applicable not only White and Red ginseng but also other processed ginseng. Furthermore it gives more information about the quality of ginseng products.

High performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) and quadrupole time-of-flight mass spectrometry (Q-TOF-MS) were used for qualitative and quantitative analysis of ginsenosides in wild *Panax* species, namely *Panax ginseng*, *Panax quinquefolius* and *Panax vietnamensis*. Identification of ginsenosides was achieved using Q-TOF-MS and contents were determined by ELSD.

The contents of 17 ginsenosides were determined by LC-ELSD. Among them 11 ginsenosides were identified using standards. Those were notoginsenoside R1, majonoside R1, ginsenoside Rg1, ginsenoside Re, majonoside R2, vina-ginsenoside R2, notoginsenoside R2, ginsenoside Rb1, ginsenoside Rc, ginsenoside Rb2, and ginsenoside Rd. To identify six other unidentified metabolites, m/z of $[M-H]^-$ and fragment ions were measured by LC-Q-TOF-MS and compared to literature values. Total content of ginsenosides in the radix and rhizome of wild *P.vietnamensis* were 111.06 and 84.44 mg/g, respectively. Total content of ginsenosides in the radix and rhizome of *P. ginseng* were 10.17 and 38.39 mg/g, respectively. Those in *P. quinquefolius* were 13.25 and 14.93 mg/g, respectively.

Peak patterns between radix and rhizome of *P. ginseng* and *P. quinquefolius* was not significantly different. But it was quite different between radix and rhizome of wild *P.*

vietnamensis. Radix contains higher amount of ginsenosides in particular due to variation in protopanaxatriol type ginsenoside contents.

Keywords: *Panax ginseng, Panax vietnamensis, Panax quenquifolius,* ginsenoside, quality control, HPLC analysis.

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1. Introduction

For many thousand years, mankind has been using various plants as nutrient, beverage, cosmetics, dye and medicine to maintain health and to improve quality of life. Ginseng is a group of plants belonging to the genus *Panax*, consisting of several species of slow−growing perennials with fleshy roots, in the family Araliaceae. Ginseng grows in Korea, China, Japan, Vietnam, and North America and is considered to be one of the most important plants in herbal medicine, with many health benefits arising from consumption of the rootand its extractives. Resembling at times a human body, ginseng roots have been considered to have wide ranging effects, benefiting the body as a whole. Ginseng has perhaps the longest continuous history of usage of any healing herb. Sometimes referred to as the "root of heaven," ginseng is a good example of an ancient herb that Western medicine is just beginning to study and understand [1]. Ginseng is characterized by the presence of ginsenosides and gintonin. The botanical/genus name *Panax* means "all−heal" in Greek, sharing the same origin as "panacea" was applied to this genus because Linnaeus was aware of its wide use in Chinese medicine as a muscle relaxant. However, true ginseng plants belong only to the *Panax* genus [2]. There are 12 recognized species and two infraspecific taxa of ginseng, depending on the method of classification. The following table contains all known species of ginseng (Table 1).

Table 1. Sepcies names of Panax genus

	Name	Rank
1	Panax bipinnatifidus Seem.	Species
2	Panax ginseng C.A.Meyer	Species
3	Panax japonicus (T. Nees) C.A. Meyer	Species
4	Panax notoginseng (Burkill) F.H. Chen	Species
5	Panax pseudoginseng Wall.	Species
6	Panax quenquifolius L.	Species
7	Panax sokpayensis Shiva K. Sharma & andit	Species
8	Panax stipuleanatus H.T. Tsai & K.M. Feng	Species
9	Panax trifolius L.	Species
10	Panax vietnamensis Ha & Grushv.	Species
11	Panax wangianus S.C. Sun	Species
12	Panax zingiberensis C.Y. Wu & Feng	Species
13	Panax bipinnatifidus var. angustifolius	Infraspecific taxon
14	Panax bipinnatifidus var. bipinnatifidus	Infraspecific taxon

Today, herbalists and physicians in the western world use ginseng to treat everything from fatigue to hypertension. Its most widely accepted and well—documented use is tied to its adaptogenic effects–its ability to enhance the body' s overall resistance to physical stress. This may include everything from increasing one' s stamina to withstanding cold temperatures. Most pharmacological actions of ginseng are attributed to ginsenosides, the major and bioactive constituents [3-5].

With the development of modern chromatography, there are many ginsenoisdes identified from ginseng up to date [5,6].On the other hand, ginseng and ginsenoisdes have been found to exhibit multiple pharmacological activities via different mechanisms and pathways in vitro, in vivo, and clinical models [4, 5,7].Having been well documented, there are hundreds of research papers as well as extensive reviews spotlighted on individual topics, that is, cardiovascular [8,9], central nervous [10], and immune systems [11,12]. Scientifically proven pharmacological effects of *Panax* ginseng are as follows: Efficacy of improving cerebral functions [13-17]. Efficacy of relieving pain [18]; Efficacy of preventing cancer and activate antitumor immunity [19-20]; Efficacy of inhibiting cancer cell growth [21.22]; Efficacy of increasing immunization functions [23,24]; Anti-diabetic efficacy [25-34]; Efficacy of improving liver functions [35-40]; Efficacy of adjusting blood pressure [41-43]; Efficacy of anti-fatigue and anti-stress [44-48]; Efficacy of improving female climacteric disorder [49];

Efficacy of improving male sexual dysfunctions [50−54]; Efficacy of inhibiting AIDS virus (HIV) growth [55−58]; Efficacy of anti−oxidation and anti−aging activity [59−66].

Ginseng saponins were isolated from the root of *Panax ginseng* and identified their structure in 1963 by Shibata et.al. [67], Shibata et.al [68], Shibata et.al [69] these saponins were called ginsenosides. Today, approximately 200 substances, such as ginsenosides, polysaccharides, polyacetylenes, peptides and amino acids, have been isolated from *Panax ginseng* [12], and more than 100 substances have been isolated from *Panax quinquifolius* and *Panax notoginseng*. Among the substances isolated from ginseng, the major and most unique components are the ginsenosides, which can be classified into 20S−protopanaxadiol−type (PPD) or 20S−protopanaxatriol−type (PPT)[70]. Dammarane−type ginsenosides are classified into protopanaxadiol and protopanaxatriol types. The protopanaxadiol−type has sugar moieties attached to OH at C−3 and/or C−20, and the protopanaxatriol−type has sugar moieties attached to OHat C−3, C−6, and/or C−20. The ocotillol−type has a five membered epoxy ring at C−20, and the oleanane−type has modified C−20 side chain. Chemically, several differences exist among *P. ginseng*, *P. quinquifolius*, and *P. notoginseng*. An important parameter used for differentiation is the presence of the ginsenoside Rf in *Panax ginseng* and *notoginseng*, the pseudo ginsenoside F11 in *P. quinquifolius* [5] and the notoginsenoside R1 in *P. ginseng and notoginseng*.

Vietnamese ginseng contains not only protopanaxadiol (PPD) and protopanaxatriol (PPT) saponins such as ginsenoside Rb1, Rd, Re, Rg1, but also ocotillol saponins, such as majonoside R1, R2 (in high yield), and vina-ginsenoside R1 and R2 [71-79]. Majonoside R2 constitutes >5% of the dried weight of VG [72].

Figure 1 shows the full structure information for the PPD, PPT, OT and OA type saponins.

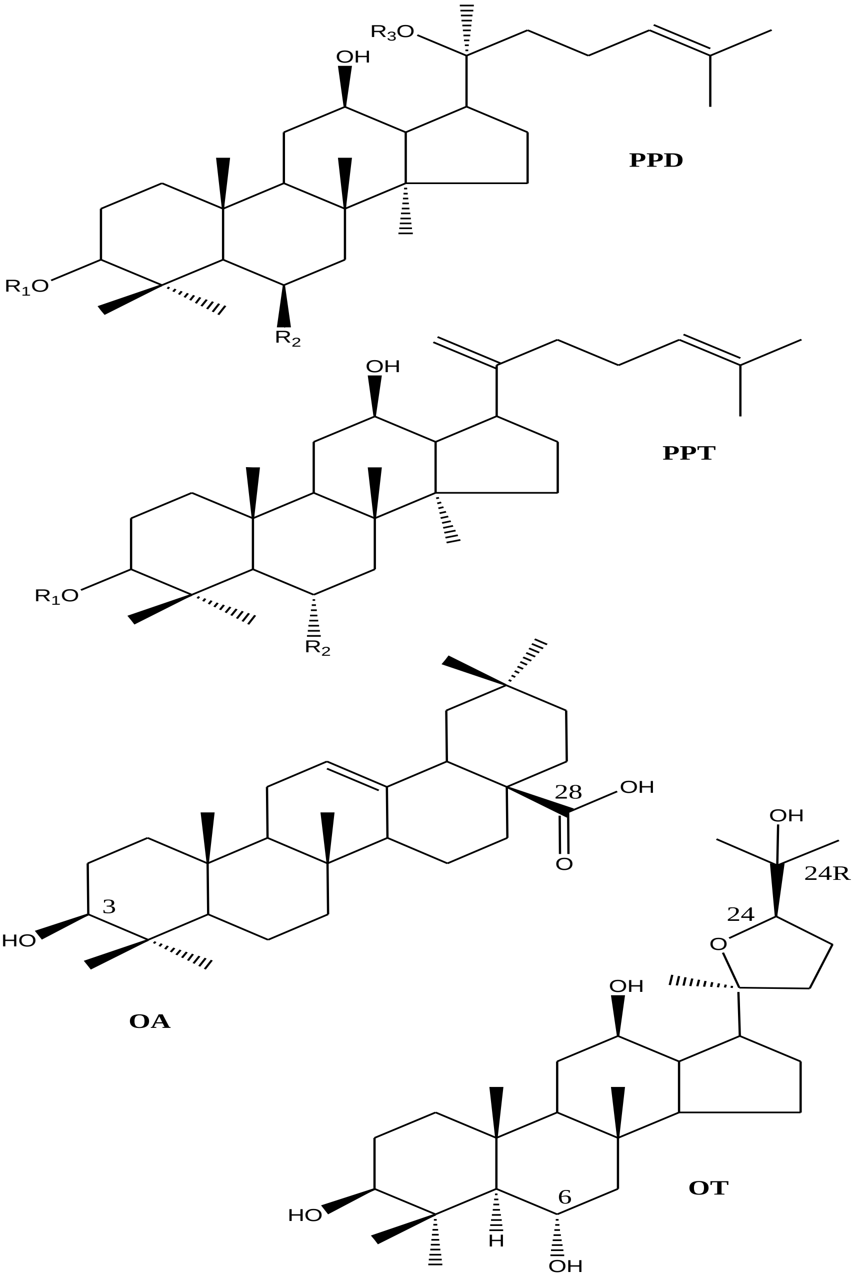


Figure 1. Structures four compounds classified into the protopanaxadiol (PPD), protopanaxatriol (PPT), octillol (OT), and oleanolic acid (OA) subtypes; Ac: acetyl

Since the first research on *P. ginseng* polysaccharide reported by Ovodov and Solov' eva [76], there were total 35 polysaccharides identified from the leaves, roots and fruits of *P. ginseng* up to date. Out of these polysaccharides, 16 ones came from the leaves, 18 ones from the roots and one from the fruits of *P. ginseng*. Flavonoids and polyacetylenes, such as kaempferol, trifolin and panasenoside, were also isolated from the roots of *P. ginseng*. Matsunaga *et al*. [77] isolated polyacetylenes, panaxynol, and panaxydol. Iwabuchi *et al*. [78] isolated two sesquierpene alcohols, pansinsanol A and panasinsanol B (Figure 2). Sesquiterpene hydrocarbons, α-panasinene, β-panasinsene, α-neoclovene, and β-panasinsene (Figure 2) were also isolated fromthe rootlet. Furthermore, two new sesquitene alcohols, ginsenol and senecrassidiol, were found [79,80]. In addition, ginsenoynes A, B, C, D, E, F, G, H, I, J, and K have been reported.

Many kinds of polysaccharides were also isolated from *P. quinquifolius*. Immunomodulating glycans, such as water-soluble COLD-FX (CVT-E002), were isolated from the roots of *P. quinquifolius* [81]. These glycans are poly furanosyl-pyranosylsaccharides. Hypoglycemic glycans, such as quinquefolans A, B, and C, were isolated from the roots of *P. quinquifolius* [82].

Polyacetylenes, such as polyacetylene PQ-1, PQ- 2, PQ-3, panaxynol, panaxydol, and 1,8-heptadecadiene-4,6-diyne-3,10-diol (Figure 3), were isolated from the roots of *P. quinquifolius* [83-85]. Nakamura *et al.* [86] isolated three flavonoids and 3 flavonoid glycosides, kaempferol 3-*O*-β-D-sophoroside-7-*O*-α-Lrhamnopyranoside, kaempferol-*O*-(2,3-di-E-p-coumaroyl-α-Lrhamnopyranoside), and kaempferol-3-*O*-α -L-rhamnopyranoside. Sanchinan-A (1.5×106), PF3111, PF3112, PBGA11, and PBGA12 were isolated from *notoginseng* [87].

Choi *et al.* [88] isolated the β-amyloid-induced neurotoxicity-ameliorating constituent, quercetin-3-β-xylopyranosyl-β-D-galactopyranoside, from the root of *notoginseng*. Wei *et al.* [89] isolated quercetin-3-O-sophoroside, kaempferol-3-*O*(2"-β-D-glucopyranosyl)-β-D-galactopyranoside, and quercetin-3-*O*(2''-β- Dglucopyranosyl)-β-D-galactopyranoside from the leaves of *P. notoginseng (Figure 4.)*. Liu *et al.* [90] isolated polyacetylene compounds, and Chan *et al.* [91] isolated trilinolein from the roots of *P. notoginseng*.

Five polyacetylenes have been isolated from *notoginseng*: panaxytriol [92], panaxynol, panaxydol [93], notoginsenic acid b-sophoroside, and 10-hydroxydeca-4,6-diynoic acid 10−O−b−D−glucopyranosyl (1 fi 2)−b−Dglucopyranoside [94]. Some of them have shown anti−tumor effects [77,95]. Several phytosterols, β−sitosterol, daucosterol [96]and stigma sterols, betulin, lupeol and calenduladiol [97]have been isolated from notoginseng (Figure 5). Phytosterols areplant−derived compounds that are similar in structureand function to cholesterol. They have properties forreducing blood cholesterol levels, and other beneficial effects for the cardiovascular system [98].Two flavonoids were isolated from notoginseng:quercetin [96] and quercetin−3−O−sophoroside [99].Flavonoids are plant phenolic compounds with significantanti−oxidant and cardioprotective effects [100].

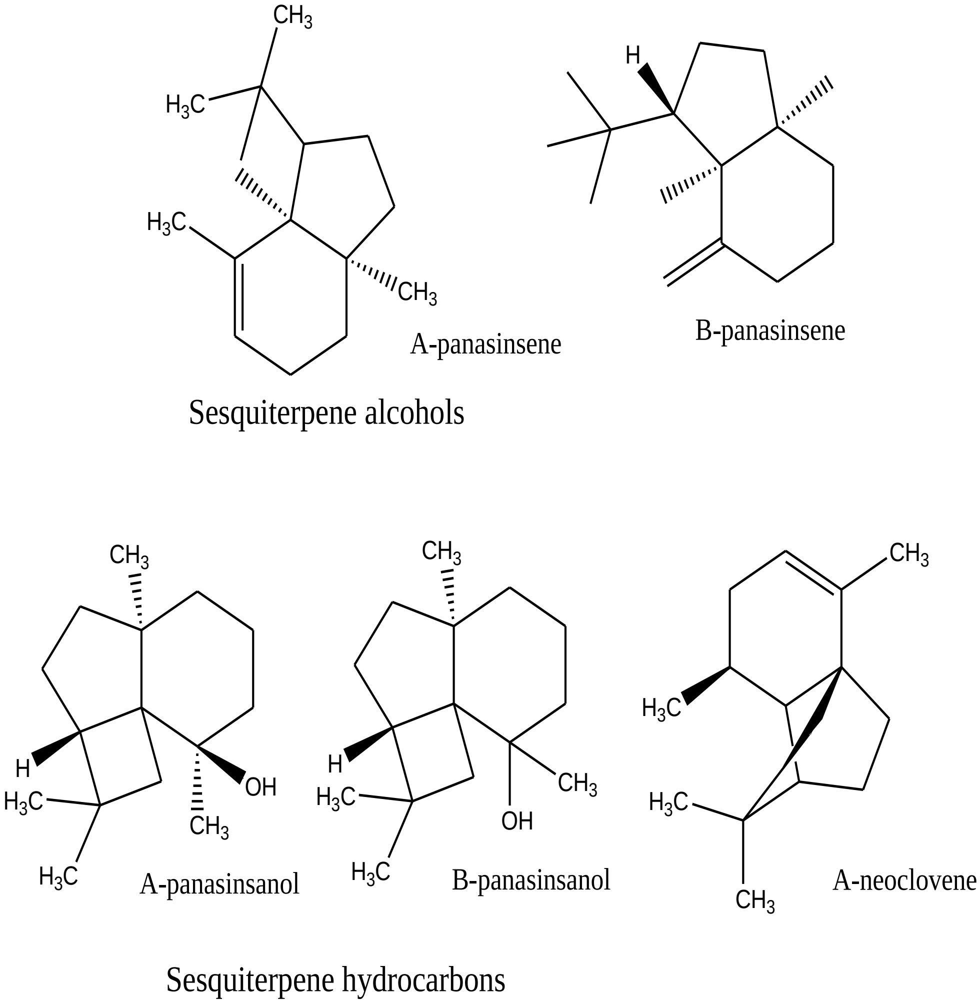


Figure 2. Sesquiterpene alcohols and hydrocarbons isolated from *Panax species*

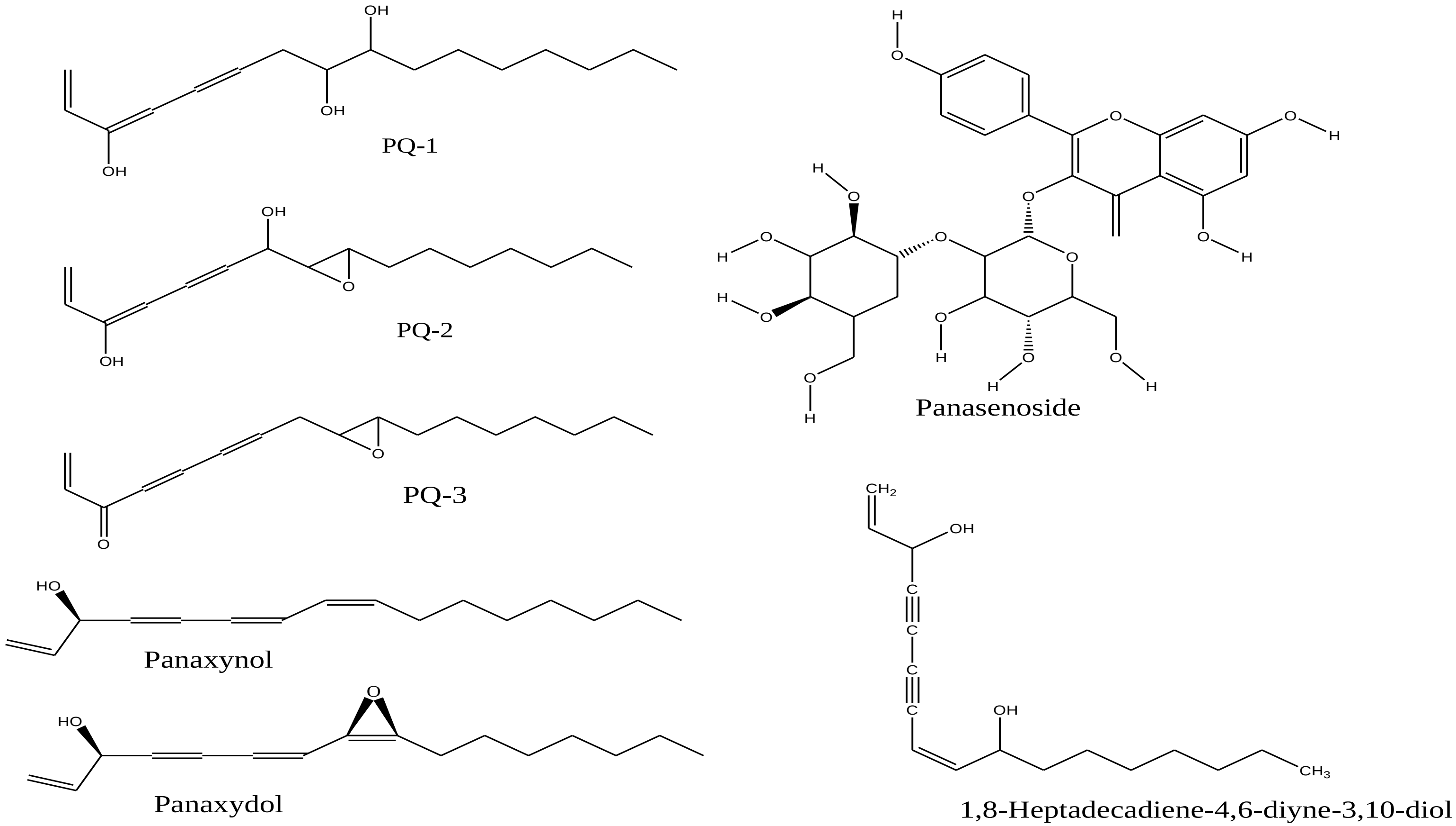
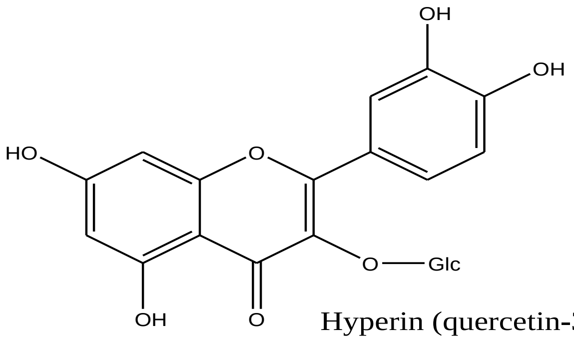
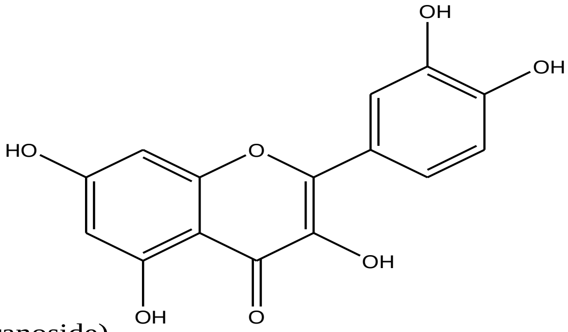


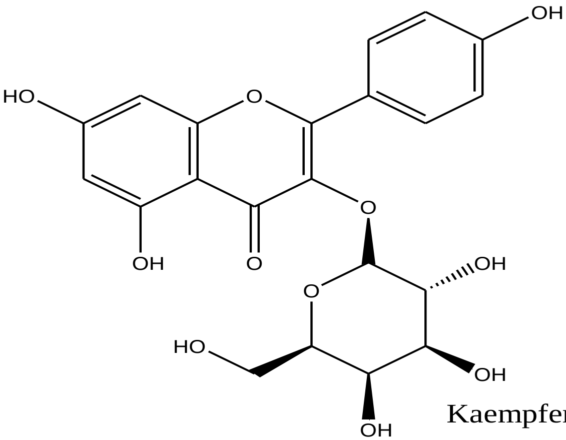
Figure 3. Polyacetylenes isolated from *Panax species*



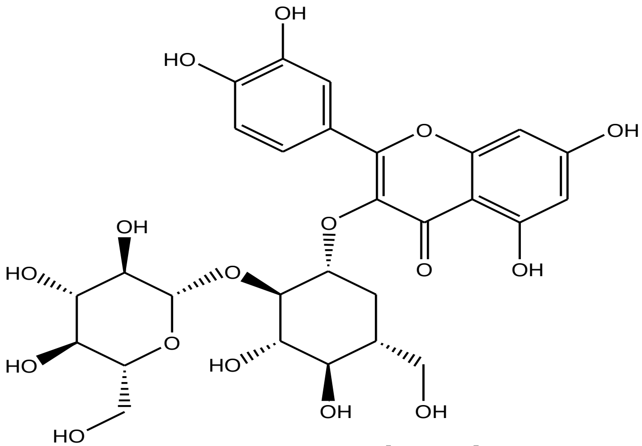
Hyperin (quercetin-3-O-β-D-galactopyranoside)



Quercetin



Kaempferol 3-O-β-D-galactoside



Quercetin 3-O-sophoroside

Figure 4. Flavonoids isolated from *Panax species*

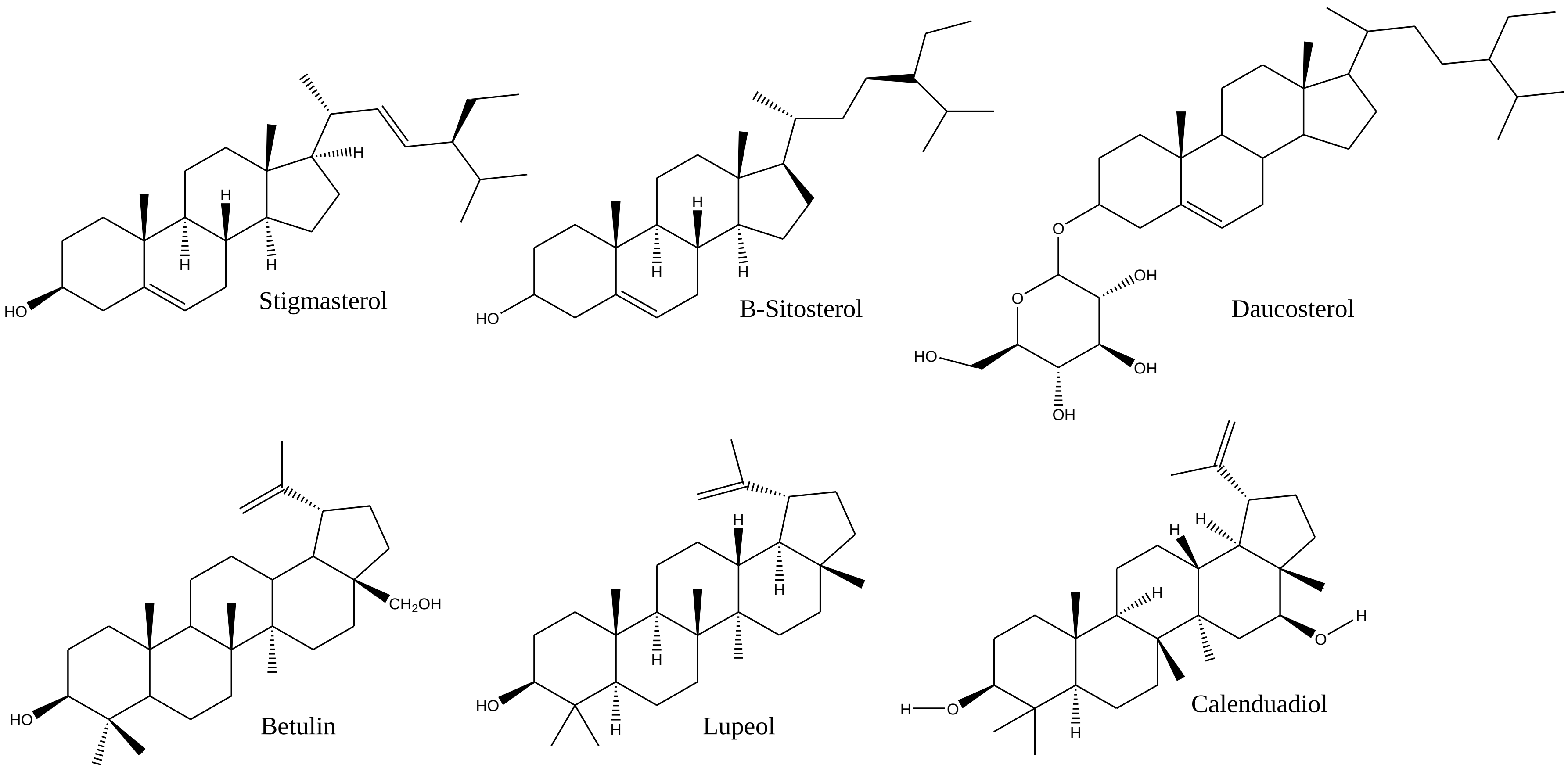


Figure 5. Phytosterols isolated from *Panax species*

The most important bioactive components of ginseng are ginsenosides, polyacetylenes, polysaccharides, alkaloide, and phenolic compounds [101]. The ginsenoside is one of the most important secondary metabolites in ginseng and various pharmacological activities. Ginsenoside has glucosyl moiety at carbon−3, −6, and −20, which can be readily converted by acid treatment and heat processing [102,103]. Therefore, many researcher have studies the aimed at converting major ginsenosides to the more active minor ginsenosides [104−107]. Steaming process is known to induce a structural change of ginsenoside and to enhance the biological activities of ginseng [108−110]. Hwang I.G. et al (2010) suggested that thermal processed Korean ginseng using water as soaking solvent is efficiency for conversion of anticancer ginsenoside, such as Rg3(S), Rg3(R), Rk1, and Rg5, from other saponins [111].

The root of *P. ginseng*is steamed and dried to prepare red ginseng, while the peeled roots dried without steaming are designated as white ginseng.

The commercially available ginseng roots are classified into two forms, red and white ginsengs. It was reported that all of the saponins found in white

ginseng were isolated in similar yields from red ginseng, while some partly deglycosylated saponins such as ginsenosides Rh1, Rh2 and Rg3 are obtained from red ginseng as artifacts produced during steaming [112]. In addition, two minor saponins were also isolated only from red ginseng, two being designated as ginsenosides Rs1 and Rs2 [113].

Park et.al., demonstrated the cytotoxicity of dammarane type glycosides from steam-processed *P. ginseng*— namely Rg3, Rg5, Rk1, Rs4, and Rs5 [114]. "Sun Ginseng" (high-pressure steam-processed *P. ginseng*) claimed the treatment to be beneficial in improving quality of life in cancer patients, mainly those suffering from gynecologic or hepatobiliary cancer[115]. Choi P. et al., (2015) developed a novel ginseng extract by microwave-assisted processing. This novel extract exhibits increased content of ginsenosides Rg3, Rg5 and Rk1[116]. Recently , same research group has investigated the protective effects of microwave-processed ginseng and its active as yet unidentified ginsenosides against cisplatin-induces nephrotoxicity in vitro and in vivo [117]. Kang K.S et al, (2010) identified the effects of heat processed giseng ant its active component 20(S)-Rg3 on the progression of renal damage in type 2 diabetic rats [118]. American red ginseng, which was treated by the steam-heat-treatment, showed the higher levels of radical scavenging activity (DPPH and superoxide anion), inhibited lipid peroxidation and increased the activity of antioxidant enzymes [119]. The important constituents in steamed American ginseng include several groups of epimers or geometric isomers, namely ginsenosides 20(S)/20(R)-Rg2, 20(S)/20(R)-Rh1, 20(S)/20(R)-Rg3, Rk3 Rh4, Rk1 and Rg5, accounting fro over 90% of total ginsenoside content [120]. Steamed *P. quinquifolius*has more potent activity than white ginseng on human cancer cells [121,122]. Steamed ginseng berry extract inhibited colorectal cancer growth both *in vitro* and *in vivo* [123]. Enhanced anticancer potential results from chemical degradation and conversion of the original saponins to new compounds during the steaming process [121,124]. Because of higher total ginsenoside concentration, American ginseng had stronger anticancer potential than Asian ginseng [125]

Steaming of *P. notoginseng*may change its composition [126-129] and alter its biological activities [130,131].Toh D. F. et al, (2011) investigate the effects of steaming on the chemical profile of raw *P. notoginseng*and the effects ofsteaming duration on anti-proliferative activities of *P.notoginseng*in three liver cancer cell lines[132].

P. notoginseng has been shown to have anticancer activity [133,134] and steaming it can increase its anticancer activity [134]Sun S. et al. (2010)analysed the influence of different steaming temperatures and duration on changes in saponin content of notoginseng root and to correlate the observed changes to antiproliferative activities in SW-480 human colorectal cancer cells[135].

Le T.H.V. et al (2014) reported that steaming *P. vietnamensis* at 120° C induces the modification of saponin constituents and enhancement of its biologicalactivity [136]. Resently they continue their study on processed *P. vietnamensis*, the processing temperature at 105_C for *P. vietnamensis* was studied on the saponins composition and antiproliferative activity[137].

Orally administered ginsenosides are very hard to breakdown by gastric juices or liver enzymes. However, ginsenosdeis are metobilized by intestinal bacteria and then these metabolites are absorbed from intestine. Many kinds of bacteria including Prevotella oris [138], Eubacterium A-44 [139], Bifidobacterium K 506 [140], Bacteroides JY6 [141], and Fusobac- terium K-60 [141] seem to cooperatively metabolize ginsenosides.

Fermented Red Ginseng was developed utilizing specific microorganism' s fermentation to increase absorption rate of ginsenoside and standardize the effect of ginsenoside [142]. By fermenting ginseng, glycoside ginsenoside is converted into non glycoside ginsenoside. In other words, glucose, attached into saponin, is metabolized by bifidus bacteria and converted into small sized ginsenoside. The fermented ginseng contains high concentration of Compound K, PPD, PPT and ginsenosides with enhanced biological activities such as Rh1, Rh2, Rg5 and Rk1. So absorption rate in the body is high and it is expected to maximize its efficiency in pharmacology [144]. Cho, H. J et al. (2010) has screened edible Lactobacillus species for their potential in metabolizing ginsenoides from red ginseng[145]. A recently isolated strain, L. plantarum M-2, that is food-grade, was used for the microbial conversion of ginsenosides in red ginseng powder [146]. Therefore, to utilize the beneficial properties of ginsenosides metabolitesusing food-compatible microorganisms, this research group investigated changes in total sugars, uronic acid,polyphenols and ginsenoside metabolites during fermentation by L. plantarum M-2. Also they investigated the anti-metastasis and immunological activities of fermented red ginseng in animal and human subjects [146,147] indicate that L. plantarum M1 is a very useful tool in the structure modification and metabolism study of ginseng, as well as for the preparation of minor ginsenosides and intestinal bacterial metabolites from ginseng powder, which possess both selectivity and efficiency.

Administration of an extract of the fermented Korean red ginseng appeared to protect liver, kidneys, heart and lungs of aged rats against oxidative stress by reducing the intensity of lipid peroxidation and by enhancing the activities of enzymatic and non-enzymatic antioxidant [148].

Compound K (Figure 6) is one of the major metabolites produced (149-152) and has been proposed as the most bioavailable metabolite produced from colonic fermentation [151]. Many different schemes have been reported to produce compound K. Compound K has been produced utilizing fungal biotransformation with Paecilomyces bainiersp. 229 [153]. Thermo-stable β-glycosides from Sulfolobus solfataricushas been reported to be able to hydrolyze ginsenosides and biotrans-form them into compound K at an optimal pH of 5.5 from a ginseng extract [154]. Conversion of both Rb1 and Rb2 to Ginsenoside Rd is one route to the production of compound K [154].Compound K produced by microbial transformation of commercial obtained ginsenoside extract with Aspergillus niger were found to inhibit the growth of a variety of cultured cells including melanoma (B16-B6, LC5012.7 μM), hepatocarcinoma (Hep-G2, LC_{50} 11.4 μM), myeloid leuke-mia (K562, LC_{50} 8.5 μM) and lung carcinoma (95-D, LC_{50} 9.7 μM) cells [155]. In human malignant astrocytoma cells, 13 ginsenosides and metabolites were screened and compound K and Rh2 were identified to induce apoptotic cell death by activating the caspases, p38, and MAPK while not affecting the growth of primary astrocytes [156]. The effects were magnified when combined with a Fas ligand which is part of the TNF/NGF receptor family [156].

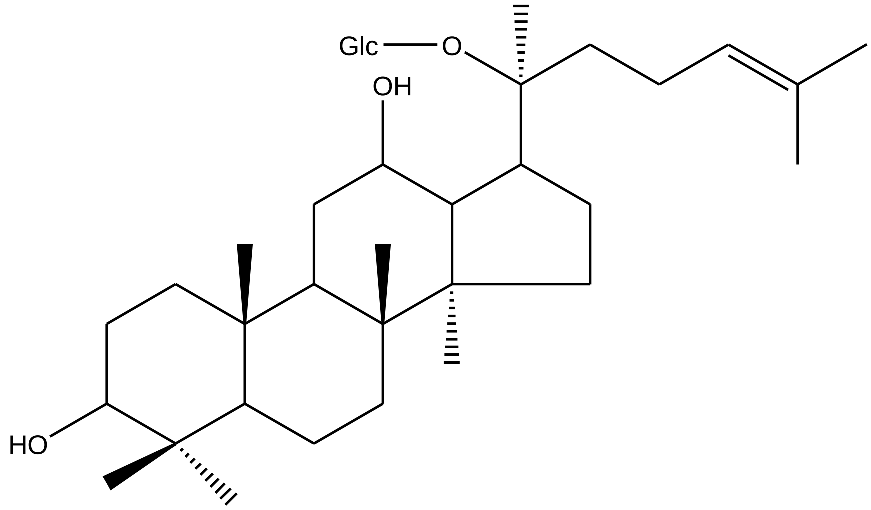


Figure 6. Compound K

Due to the fact that Ginseng is a very popular phytomedicine used all around the world, a huge quantity of work has been carried out during the last 40 years in order to develop analytical methods for the identification, quantification and quality control of ginsenosides in raw plant materials, extracts and marketed products [157, 158]. TLC is a very common technique for the fingerprint analysis of plant material and extract due to its easiness of use, low cost and versatility. Asian and American ginseng can be discriminated for their ginsenosides composition by two-dimensional TLC [160]. With the introduction of densitometry, TLC has become also a useful tool for the quantitative analysis: Ginsenosides of *P. ginseng* roots and preparations can be quantified using HPTLC Silica gel F_{254} [161]

HPLC is fast, sensitive and can be applied to non-volatile and thermally labile compounds. Another advantage is versatility due to the possibility of using different detection techniques such as an ultraviolet detector (UVD), evaporative light scattering detector (ELSD), charged aerosol detection (CAD), fluorescence detector (FLD), pulsed amperometric detector (PAD), and a mass spectrometer. Most HPLC applications in ginseng analysis over the past 5 years have involved the quantitation of ginsenosides or related projects [162].

Among the different detection techniques of ginsenoside analysis, the UV is the most employed detector found in phytochemical laboratories. Because of the weak UV absorption of ginsenosides, their detection is usually achieved at 198–205 nm. The great majority of the literature methods use C_{18} columns with water or phosphate buffers and acetonitrile mixtures as solvent system either in isocratic or in gradient elution mode [163-170]. The concentration of the phosphate buffer has been shown to be important in order to obtain separation of twelve ginsenosides in white *P. ginseng* extracts [171]. The main problems encountered in performing HPLC-UV analyses of ginseng are the high level of baseline noise and the poor sensitivity due to the weak UV absorption of ginsenosides. This feature also limits the choice of solvents and mobile-phase modifiers for improved separation. ELSD is a mass detector which measures the scattered light generated by the non-volatile particles of analyte produced by the nebulization into droplets of the LC effluent. The signal intensity is related to the concentration of the solute in the effluent but not its chemical identity. ELSD is a universal, non-specific detector which can provide a stable baseline even with gradient elution. Furthermore, many volatile mobile phase mofidiers are available to obtain better resolution and selectivity [157,172]. Park M.K et al. was the first to use ELSD to quaintify ginsenosides on an amino-bonded column [173]. Yun BS et al. the same research group developed a new HPLC-ELSD method for the determination of less polar ginsenosides in processed *Panax ginseng* [174]. Since then, ELSD has been consideredthe standard choice for ginsenoside analysis. The HPLC-ELSD method has primarily been used for the simultaneous quantitation of various ginsenosides from

Panax species or transformed ginseng preparations [175–179], and a principal component analysis of HPLC–ELSD chromatograms has been used to discriminate the geographic origins of *P. ginseng* roots [180].

CAD was developed as an alternative to ELSD to detect poor UV–responsive analytes. CAD is a mass–selective detector for non–volatile compounds, and its major advantages over ELSD are enhanced sensitivity and reproducibility. Using an HPLC–CAD system, Bai *et al.* [181] and Wang *et al.* [182] simultaneously quantified ginsenosides in *P. ginseng* and *P. notoginseng*.

Fluorescence is one of the most sensitive detection methods for HPLC analyses. A HPLC method using photoreduction fluorescence detection was described for the analysis of ginsenosides Rb1, Rb2, Re, Rd and Rg1 in *P. ginseng* and *P. notoginseng* [183,184]. A novel precolumn derivatization method for the quantitative determination of ginsenosides Rb1 and Rg1 by HPLC with fluorescence detection.was developed by D. Shangguan et al,[185]. PAD is a less popular HPLC detector in ginseng analysis. It is an electrochemical detector that measures the positive potential produced by sample oxidation on a gold electrode, lending itself to carbohydrate or polyalcohol quantitation coupled with high–performance anion–exchange chromatography (HPAEC). Park MK et al.

(1994) was the first to develop an ion chromatography-PAD method for ginsenoside analysis [186]. Joo *et al.* [187] simultaneously determined two Amadori compounds in Korean red ginseng and plasma samples from rats treated with red ginseng extract using HPAEC-PAD. Kwon *et al.* [188] and Kwon *et al.* [189] developed a method for the highly sensitive quantitation of ginsenosides using reversed-phase HPLC-PAD under alkaline conditions. UPLC, an advanced type of HPLC, has emerged as a powerful tool in many analytical laboratories to profile phytochemicals in crude plant extracts [190]. Dan *et al.* [191] and Xie *et al.* [192] developed an UPLC-electrospray (ESI)-MS method to determine saponins in *P. notoginseng*, and an UPLC time- of-flight (TOF)-MS for metabolomics profiling of several *Panax* plants. GC is a high-resolution and, environment friendly separation technique that avoids the use of toxic solvents. Volatile compounds in ginseng have easily been determined by GC with applicable detection technologies, such as MS. Xie *et al.* [193] determined volatile oils in the root of *P. ginseng* at different ages. Abd El-Aty *et al.* [194] characterized volatile flavor compounds with GC-MS and determined their content in white and red ginseng. Liu *et al.* [195] determined polyacetylenes in *Panax* species using GC-MS method. MS-based applications have occupied a major portion of ginseng analyses in recent years and are leading the trend in analytical methods. Because of its powerful identification and quantitation capabilities, MS is a versatile analyzer for the analysis of non-volatile molecules in ginseng, such as ginsenosides and their metabolites in biological systems [157,172,196]. Various types of mass analyzers are used for ginseng analysis: quadrupole (Q), ion trap (IT), TOF, and Fourier transform ion cyclotron resonance (FT-ICR) [172]. Moreover, multi-stage MS combines different mass analyzer designs that give advanced structural information, sensitivity, specificity, and versatility [172]. Triple quadrupole (QqQ), Q-IT, Q-TOF, or IT-TOF are used for ginseng analysis. Quadrupole is the simplest and least expensive mass analyzer. TOF analyzer measures accurate mass with high resolution and a full-scan mass range, thereby making it suitable for the identification of unknown metabolites.A variety of extraction, purification, and analysis procedureshave been utilized to obtain and quantify ginsenosides fromleaves [197,198], berries [199], or most commonly, roots [200-203]. Most extraction procedures *use methanol* or ethanol [201,202] as solvents, either pure or as an aqueous solution [201], and solvents often accompanied by heating, refluxing, or sonication[200-205]. Subsequent purification can include cloud-point extraction/salting out [206], oxidative cleavage and trimethylsilylation [204], solid-phase extraction [206,207,208], or additional extraction with diethyl ether/n-butanol [163,197, 209, 201-213].Undoubtedly, ginseng saponin is the main constituents of ginseng and they are used as markers for the quality control of ginseng. In Korean Pharmacopeia (KP) and Japanese Pharmacopeia (JP), ginseng is defined as the root of *P. ginseng* which contains not less than 0.10% of ginsenoside Rg1 and not less than 0.20% of ginsenoside Rb1(Table 2 and Table 3). In Chinese Pharmacopeia (CP), ginseng should contain not less than 0.30% of Rg1 + Re and not less than 0.20% of Rb1.This kind of standard is appropriate for the quality control of white ginseng and its powder. However, some ginseng products are difficult to apply this standard as ginseng saponin is not stable in some environment. It is well known that most ginsenoside lose a part of its sugar moiety and transformed to less polar ginsenoside especially under the heat, in acidic condition, or by fermentation. Heating process converts the naturally occurring ginsenoside Rb1 and Rg1 into artifact ginsenosides such as ginsenoside Rg3, Rg5, Rh1, and Rh2, among others. Therefore, ginsenoside Rg1 and Rb1, which have been used as quality markers of ginseng, do not express the quality of some ginseng products. The analytical methods currently used to detect these two compounds not appropriate by several parameters for the analysis of processed ginseng: 1) ginsenoside Rg1 and Rb1 are just two of several saponins present in ginseng; 2) the contents of the two ginsenosides are not necessarily proportional to the amount of total saponins; 3) this kind of standard is appropriate for the quality control of white ginseng and its powder, but does not express the quality of some ginseng products; 4) multiple runs or strict analytical conditions are needed in the HPLC analysis because ginsenoside Rg1 and Re are difficult to separate. Considering many kinds of processed ginseng and its products were developed recently, it is necessary to develop a new standard for the quality control of ginseng products. It is generally known that purchasing or isolation of ginsenosidesis time, labor, and money consuming. Therefore, the goal of this experiment is developing a new simplified and inexpensive analytical method for all kinds of ginseng products with the using of reference standards and employing a single chromatographic step.

Table 2. Comparison of Korean Official Compendia for Ginseng

Compendium	**Items listed**	**Criteria**
Korean Pharmacopeia (KP)	Ginseng	Rg1 : ≥ 0.10%, Rb1: ≥ 0.20%
	Red Ginseng	Rg1 : ≥ 0.10%, Rb1: ≥ 0.20%
Korean Herbal Pharmacopeia (KHP)	Ginseng Powder	Rg1 : ≥ 0.10%, Rb1: ≥ 0.20%
Health Functional Food Code	Raw Material for Ginseng Health Functional Foods	Rg1 + Rb1 : 0.8-34 mg/g
	Raw Material for Red Ginseng Health Functional Foods	Rg1 + Rb1 + Rg3: 2.5–34 mg/g

Food Code	Ginseng / Red Ginseng Drink	Ginseng Components : ≥ 0.15%

Table 3. Comparison of Quality Standards of Ginseng in the World

Country	Items	Criteria
Korea (KP)	Ginseng / Red Ginseng	Rg1 ≥ 0.10%, Rb1≥ 0.20%
Japan (JP)	Ginseng / Red Ginseng	Rg1 ≥ 0.10%, Rb1≥ 0.20%
China (CP)	*P. ginseng* Leaves	Rg1 + Re ≥ 2.25%
	P. ginseng Roots	Rg1 + Re ≥ 0.30%; Rb1 ≥ 0.20%
	Red Ginseng Roots	Rg1 + Re ≥ 0.25%; Rb1 ≥ 0.20%
	P. notoginseng Roots	Rg1 + Re + NR_1 ≥ 5%
	Saponins from *P. notoginseng*	NR1≥ 5%; Rg1 ≥ 25%; Re ≥ 2.5%; Rb1 ≥ 30%; Rd ≥ 5%
USA (USP)	*P. quinquefolius* Powder	Rg1 + Re + Rb1 + Rc + Rb2 + Rd ≥ 4%
	P. quinquefolius Extract	Rg1 + Re + Rb1 + Rc + Rb2 + Rd ≥ 10%
	P. ginseng	Rg1 ≥ 0.10%, Rb1≥ 0.20%

	P. ginseng Extract	Rg1 + Re + Rb1 + Rc + Rb2 + Rd ≥ 3%
EU (EP)	Ginseng	Rg1 + Rb1 ≥ 0.40%
CODEX	Ginseng Products (Food)	Detection of Rb1 Detection of Rf for *P. ginseng*

2.Experimental

2.1. Materials

Ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, Rd, Rg_3(S), Rk_1 and Rg_5were isolated in our laboratory from heat processed ginseng using open-column chromatography and semi-preparative liquid chromatography (Figure7).The purity of all ginsenosidestandards was determined to be over 99% by LC-ELSD analysis. Standard stock solutions were prepared in methanol and stored at 4° C before use. Working solutions were freshly made by diluting the stock solutions with methanol. Notoginsenoside R_1 and R_2 were purchased from Ambo Institute (Daejeon, Korea). Majonoside R_1, R_2, and Vina-ginsenoside R_2 were provided by the University of Medicine and Pharmacy, Ho Chi Minh city, Vietnam. The identity and purity of these standards were confirmed by LC-UV-ELSD and LC-MS. HPLC-grade methanol, acetonitrile, and water were purchased from J. T. Baker (Phillipsburg, New Jersey, United States).

Fresh roots of *Panax ginseng* were purchased from a local market in Seoul, South Korea.

Wild *Panax vietnamensis* was collected from Yunnan province, China. Wild *Panax ginseng* and *Panax quinquefolius* were obtained from Manchuria, China and Ontario, Canada, respectively.

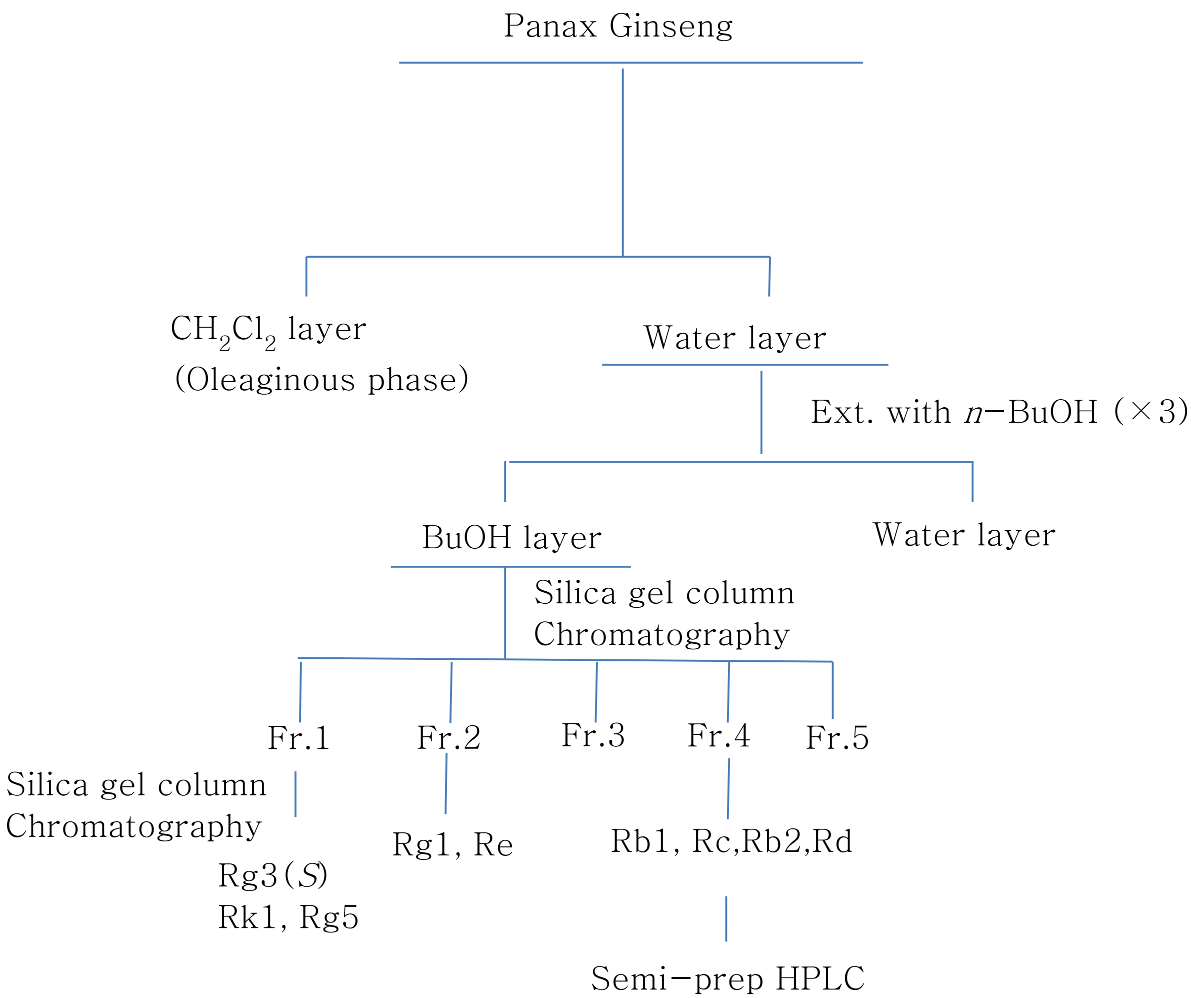


Figure7. Isolation of ginsenosides from heat-processed ginseng

2.2. Instruments

2.2.1. LC−UV

High−performance liquid chromatographic analysis was carried out on a Perkin-Elmer (Waltham, Massachusetts, United States) LC Series 200 instrument consisting of an LC pump, a UV/VIS detector, a vacuum degasser, and an autosampler fitted with a 200 μL sample loop. A C18 column with a particle size of 5 μm, an inner diameter of 4.6 mm, and a length of 250 mm was purchased from Waters (Mildford, Massachusetts, United States) and used at room temperature.

2.2.2. LC−ELSD

High−performance liquid chromatographic analysis was carried out on a Perkin-Elmer (Waltham, Massachusetts, United States) LC Series 200 instrument consisting of an LC pump, a vacuum degasser, and an autosampler fitted with a 200 μL sample loop, and Alltech ELSD 2000

(Deerfield, Illinois, United States) was used to quantitate ginsenosides in ginseng species.

2.2.3. LC−Q−TOF−MS

A Waters ACQUITY UPLC (Massachusetts, United States) combined with a 'micrOTOF−Q II' (Bruker Daltonik Inc. Bremen, Germany) mass spectrometer was utilized to identify ginsenosides, including unidentified ones. A Waters ACQUITY BEH C18 column, 2.1 mm I.D.×100 mm L, 1.7 μm particles was used for separation.

2.3. Methods

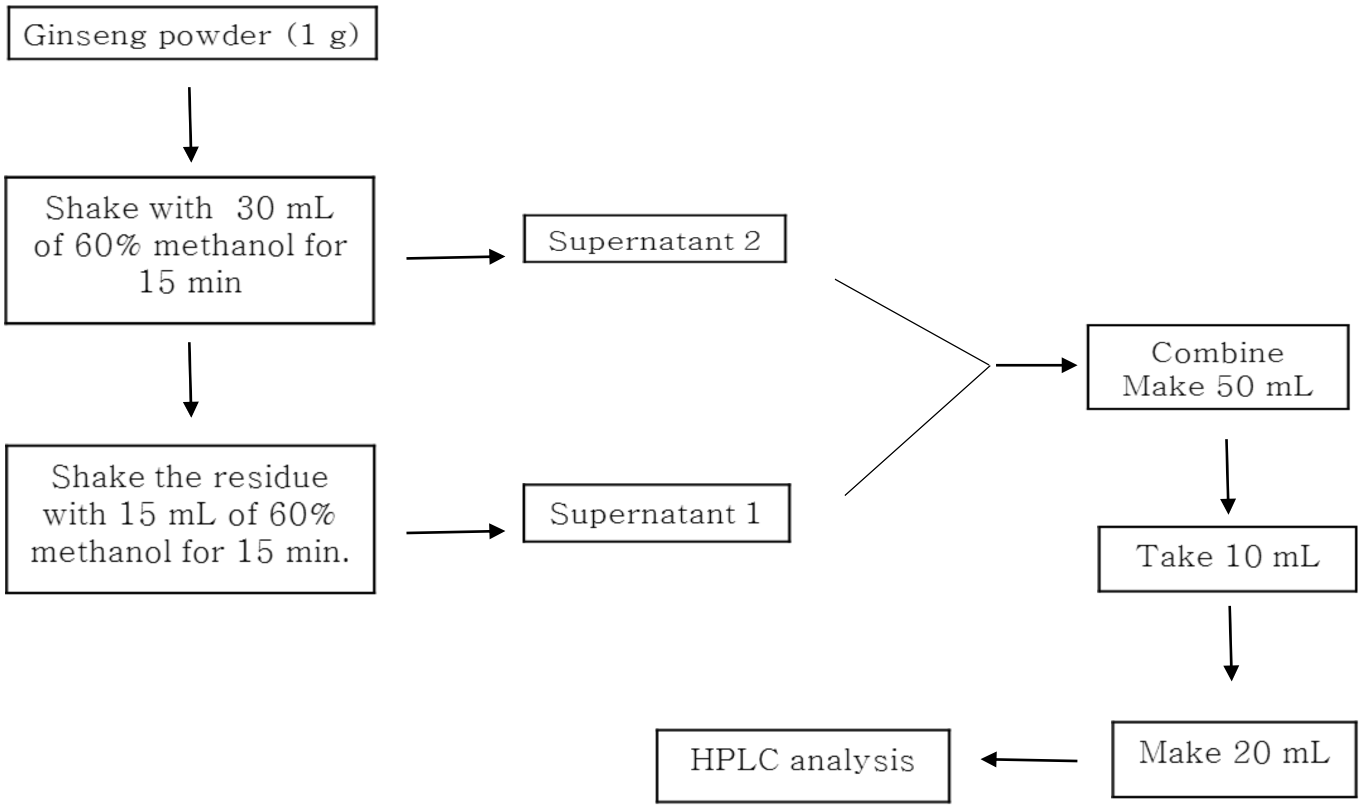
2.3.1. Sample preparation of heat treated ginseng and *Panax* ginseng

Heat-treated ginseng was prepared by steaming *P. ginseng* at 120° C in an autoclave for three hours and drying in an oven. White ginseng powder was prepared by pulverizing the purchased ginseng roots. Heat-treated ginseng powder was prepared in the same fashion. Five solvent systems for extraction were prepared: 50, 60, 70, 80, and 90% (v/v) methanol in water.

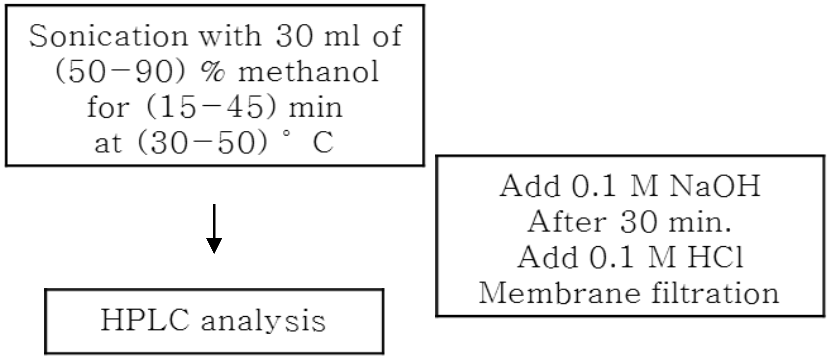
An approximately 1 g of each powder sample was added to 30 mL of one of the five solvent systems and sonicated at 30-50° C for 15-45 min in a Branson (Danbury, Connecticut, United States) 5510 ultrasonic bath. A small amount of sodium hydroxide was added to the volumetric flask for hydrolysis. The solution was neutralized with hydrochloric acid, and the solvent for extraction was added until the meniscus rested on the graduation mark. The solution was filtered through a syringe filter unit with a pore size of 0.45 μm, purchased from Advantec MFS, Inc. (Dublin, California, United States).

2.3.2. Preparation of standard solution

Approximately 1 mg of each standard was weighed and transferred into a vial, and methanol was added to yield a 1 g/L stock solution. Then, the stock solution was diluted stepwise to yield five standard solutions whose concentrations are from 20 to 320 mg/L in a geometric fashion. Each standard solution was filtered through a syringe filter unit with a pore size of 0.45 μm.



Scheme 1. Sample preparation for the analysis of ginseng in Korean Pharmacopeia



Scheme 2. Proposed method for the sample preparation of ginseng

2.3.3. Sample preparation of wild *Panax* species

The dried radix and rhizome of three wild *Panax* species (*Panax vietnamensis*, *Panax ginseng*, and *Panax quinquefolius*) were pulverized. Dried powder (150 mg) was extracted sequentially in 10,10, and 5 mL of methanol by sonication for 60 min at 50 ° C. After extraction, supernatants from centrifugation (3,000 rpm, 5 min) were collected in volumetric flasks and diluted to 25 mL. The collected solution was concentrated and reconstituted as appropriate, then filtered with a 0.2 μm syringe filter prior to injection.

2.4. Chromatographic conditions

2.4.1. LC-UV analytical condition

The eluent was initially held at 21% acetonitrile for 10 min, then ramped to 24% acetonitrile over 15 min, ramped to 33% acetonitrile over 5 min, ramped to 37% acetonitrile over 12 min, then ramped to 80% acetonitrile over 27 min, and finally held for 5 min. The total run time was 74 min, which did not include the time for equilibrium; the initial condition was held for 10 min right before each injection. The flow rate was set at 1.0 mL/min, and the injection volume was 20 μL. The UV/VIS detector was set at 203 nm.

2.4.2.LC-ELSD analytical condition

Chromatographic separation was performed on a Waters (Massachusetts, United States) sunfireC_{18} column, 4.6 mm I.D. ×250 mm L, packed with 5 μm particles at 30 ° C. The mobile phase consisted of water (phase A) and acetonitrile (phase B). The flow rate was 1 mL/min gradient elution was used as follows: 0-1 min (18% B), 1-5 min (18-25% B), 5-25 min (25-32% B), 25-29 min (32% B), 29-40 min (32-38% B), 40-45 min (38-45% B), 45-46 min (45-100% B), 46-56 min (100% B). 10 μL of sample solution was injected for analysis.

ELSD settings were optimized as below: evaporation temperature 80 ° C, nitrogen gas flow 2.0 L/min, and detector gain 16.

2.4.3.LC−Q−TOF−MSanalytical condition

Mobile phase consisted of water with 0.1%formic acid (phase A) and acetonitrile with 0.1% formic acid (phase B). The flow rate was 0.3 mL/min and the gradient condition was as for LC−ELSD analysis. 5 μL of sample solutions were analyzed. The ‘micrOTOF−Q II’ parameters were set as follows: negative ion mode, capillary 3.5 kV, nebulizer pressure 1.2 bar, dry gas flow rate 8 L/min, and temperature 200 ° C. The following ion transfer and collision stages were used: funnel 1 RF 400 Vpp, funnel 2 RF 400 Vpp, hexapole RF 400 Vpp, quadrupole ion energy 15 eV, collision energy 10 eV, collision RF 400 Vpp, transfer time 100 μs, prepulse storage 5 μs. High purity nitrogen and argon were used for nebulization and collision, respectively.

2.5. Optimization of the extraction conditions

In order to obtain quantitative extraction, variables involved in the procedure such as extraction solvents (Figure 8),(Figure 9) and temperature were optimized (Figure 10). Five different solvent conditions including 50, 60, 70, 80, and 90% (v/v) methanol in water were tested for the efficient extraction of ginsenosides from two ginseng samples, White ginseng and heat−processed ginseng. Aqueous methanol solutions were tried as the extraction solvent. As shown in Figures 8 and 9 no significant differences were obtained in solvent extractions and temperature. It could be seen from Fig. 10 that 70% aqueous methanol extraction provided high extraction yield. Thus 70% aqueous methanol was chosen as the favorable extraction solvent.

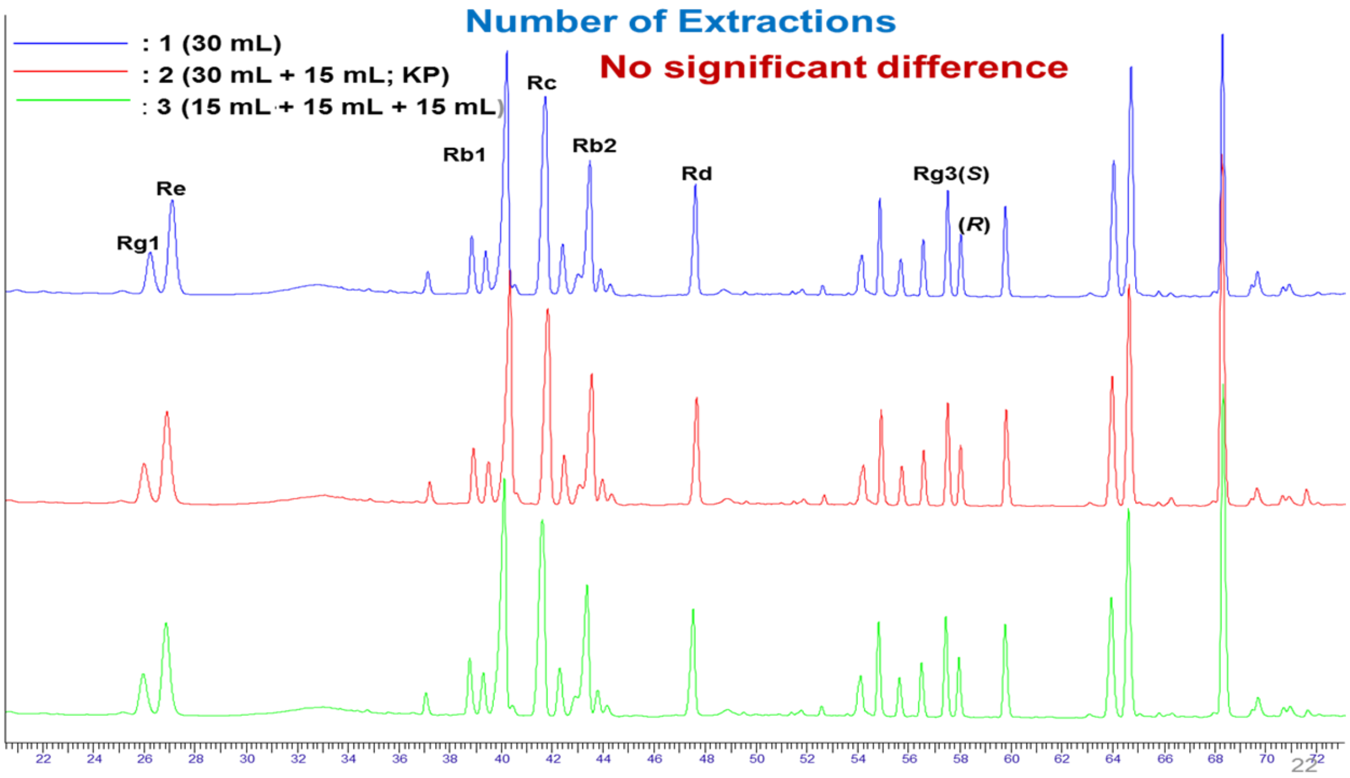


Figure 8. Effects of number of extractions on the extraction. 1) Proposed method 2) Official method (KP) 3) Threefold extraction

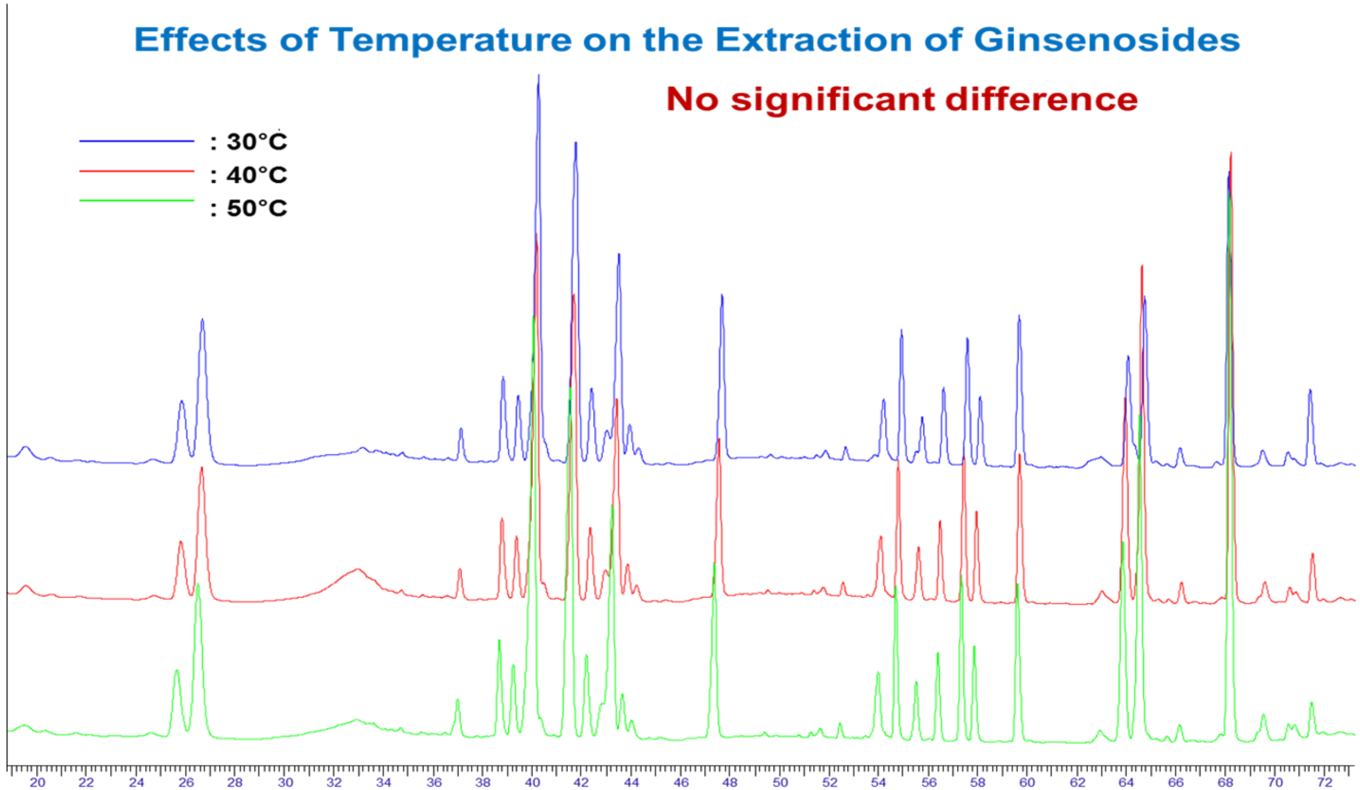


Figure 9. Effects of temperature on the extraction 1) 30° C 2) 40° C 3) 50° C

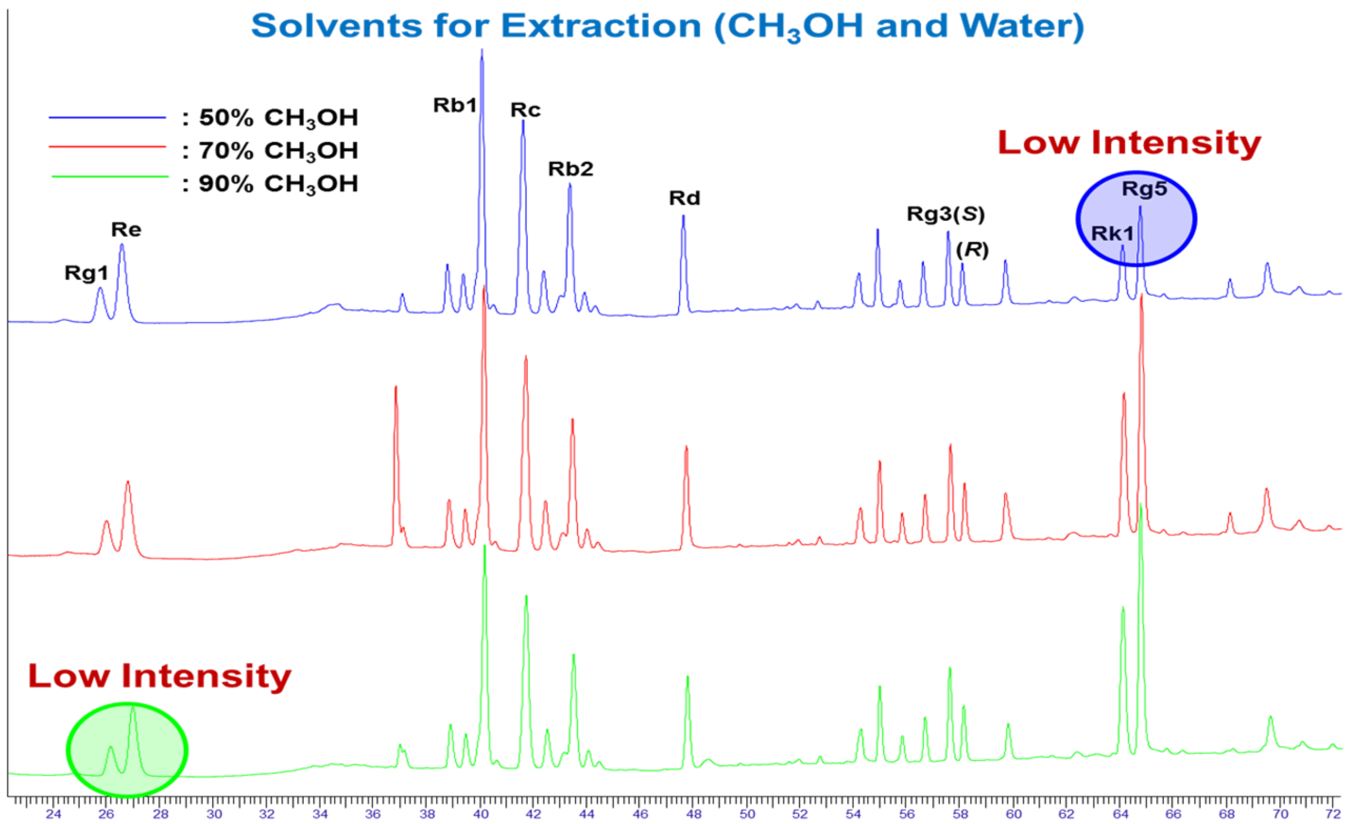


Figure 10. Effects of extraction solvents on the extraction (methanol and water)

2.6. Quantification of ginsenosides

The linearity, regression and linear ranges for 9 ginsenosides were determined using the developed HPLC−UV method. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as measure of sensitivity. The LOD and LOQ under the present chromatographic conditions were determined on the basis of response at a signal−to−noise ratio (S/N) of3and 10, respectively.

3. Results and discussion

3.1.Optimization by Design of Experiment

In general usage, design of experiments (DOE) or experimental design is the design of any information-gathering exercises where variation is present, whether under the full control of the experimenter or not. [41]

To study the effects of parameters (extraction time, solvent type and temperature) on the extraction of *Panax* ginseng a Box-Behnken design was implemented. [42]

Thirteen runs were carried out to cover all possible combination of the three factors. All runs were randomly performed with three replicates.

The results of design with three factors, extraction time, extraction solvent (five levels) and temperature (three levels) are given in Figure 11, 12 and 13.

Generally, ginsenosides can be extracted with a variety of solvents but hot water, methanol and ethanol are typically used. Methanol extractions tend to extract a greater abundance of ginsenosides.

The effects of the solvent polarity on the extraction of the nine ginsenosides are crucial, temperature is much less significant. The optimal condition was 73% CH_3OH, 41° C (Figure 11). Both temperature and time has a little influence on the extraction of ginsenosides. The optimal condition was 41° C, 28 min (Figure 12). When the time is much less significant, the effects of solvent polarity for the extraction of ginsenosides are crucial (Figure 13).

To this end, nine ginsenosides, Rb1, Rb2, Rc, Rd, Re, Rg1, (20*S*)-Rg3, Rg5, and Rk1, extracted from the two ginseng preparations were analyzed by a 74-min chromatographic method. Unlike most reported conditions, however, in our method, the analytes were eluted at a flow rate of 1 mL/min and detected by a UV spectrophotometer at 203 nm, allowing for more universal applicability. While most ginsenosides were well separated from one another, the resolution between the first two saponins peaks in the chromatograms, corresponding to ginsenosideRg1 and Re, respectively, was approximately 1.2.

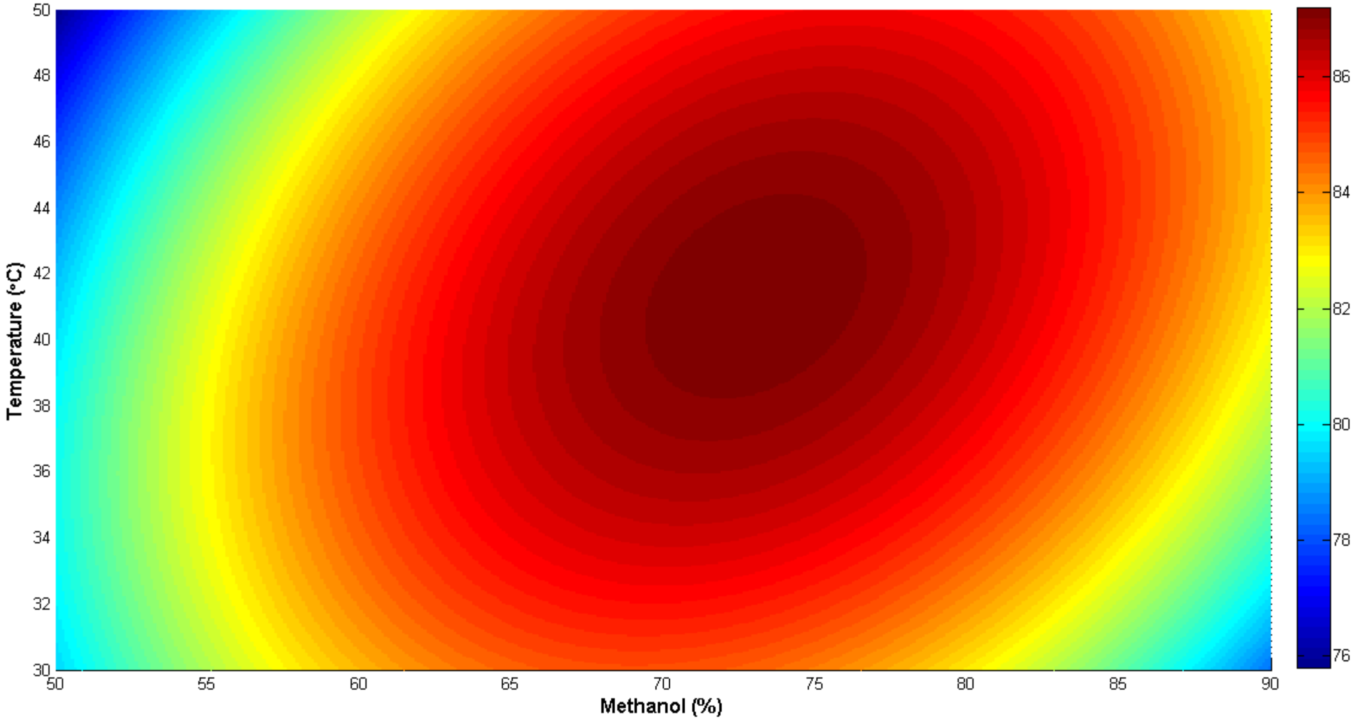


Figure11.Effects of solvents and temperature on the extraction

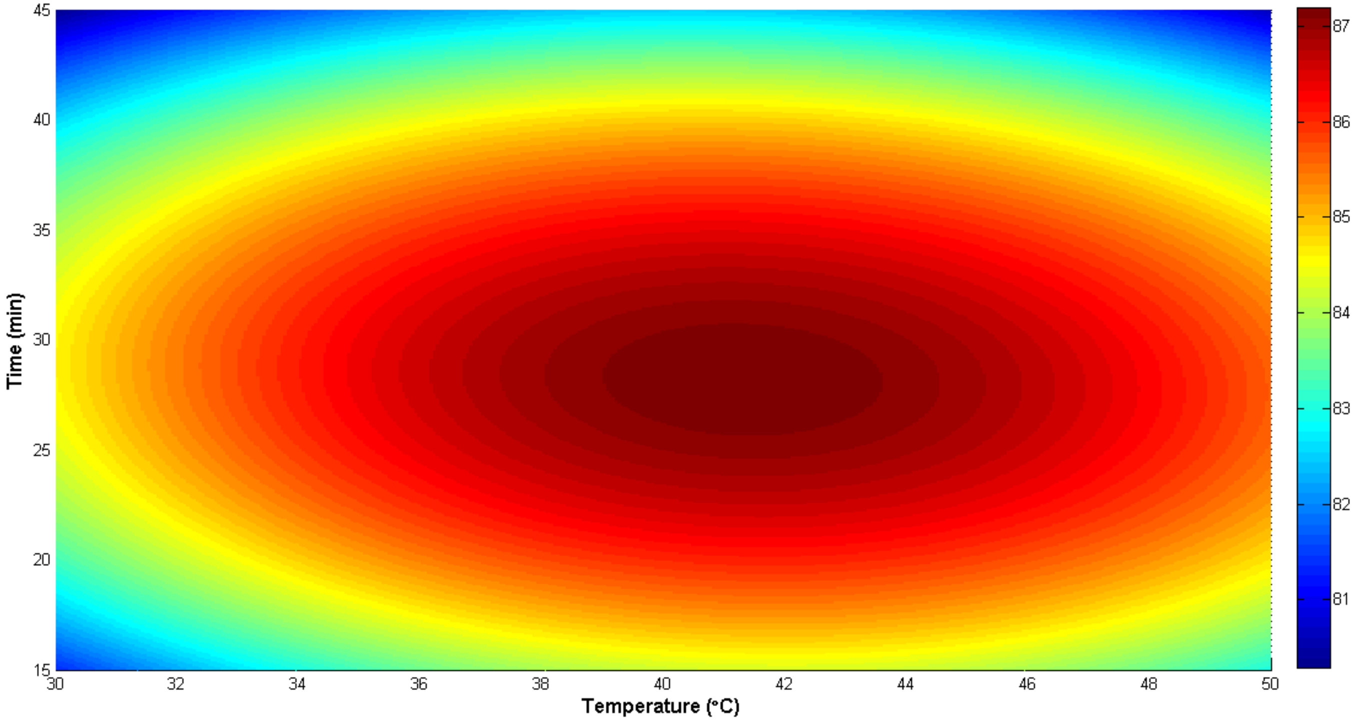


Figure 12. Effects of temperature and time on the extraction

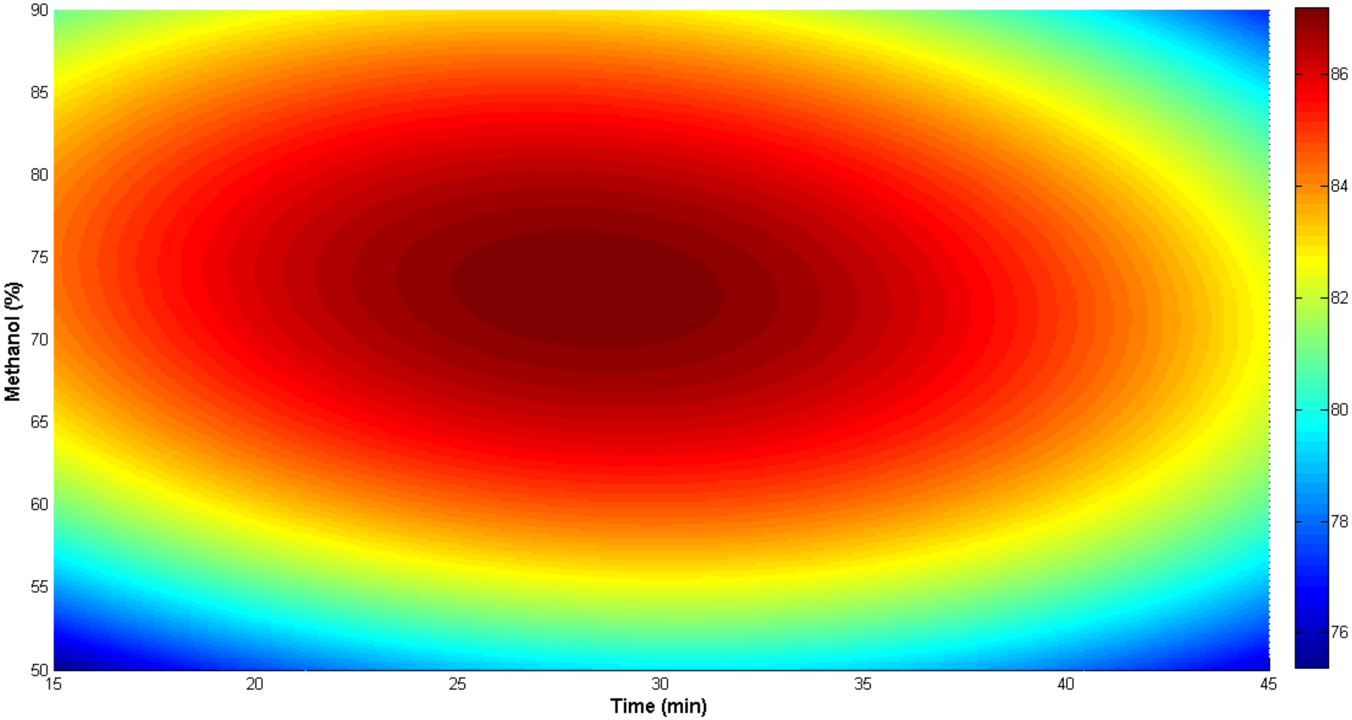
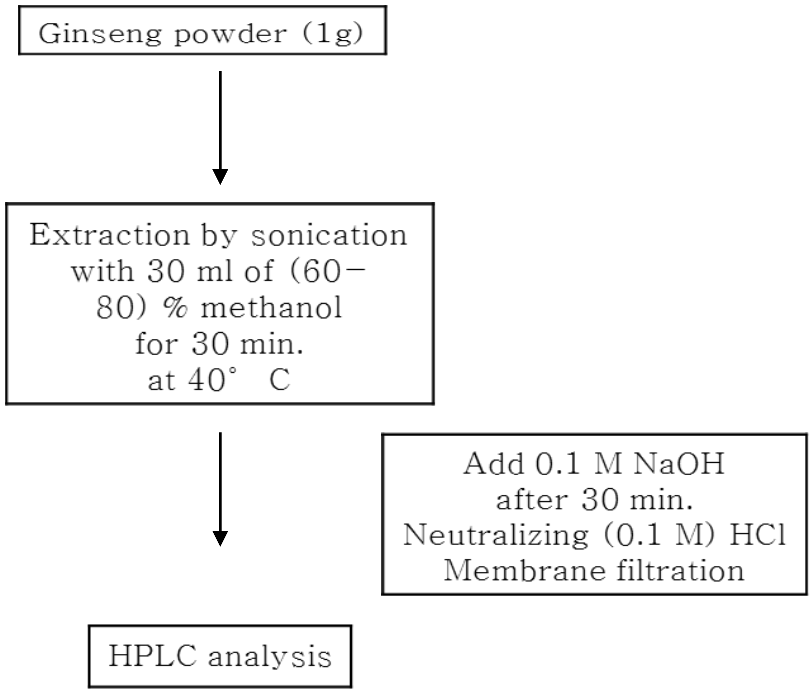


Figure 13. Effects of Time and Solvents on the Extraction

3.2.Quantification of ginsenosides

The calibration curves, the LODs and LOQs for the ginsenosides are shown in Table 4. The correlation coefficient ($R^2 \geq 0.09942$) values indicated the appropriate correlations between concentrations of investigated compounds and their peak areas within test ranges. LODs, of 1 – 7 ng/ml and LOQs of 2 to 23 ng/ml were achieved.



Scheme 3. Proposed method for the sample preparation of ginseng

Table 4. Calibration curves, squared correlation coefficients, test ranges, LODs and LOQs of nine ginsenosides

Ginsenoside	Calibration curve	R^2	Test range (mg/L)	LOD (ng)	LOQ (ng)
Rg1	y = 241x + 344	0.9999	20–320	5	17
Re	y = 176x + 311	0.9999	20–320	7	23
Rb1	y = 333x + 2041	0.9986	20–320	3	9
Rb2	y = 262x − 73	0.9962	20–320	3	11
Rc	y = 274x + 2719	0.9961	20–320	4	15
Rd	y = 334x + 2044	0.9985	20–320	3	8
(20*S*)−Rg3	y = 595x − 1370	0.9980	20–320	1	5
Rk1	y = 946x + 11327	0.9942	20–320	1	3
Rg5	y = 1587x + 4289	0.9997	20–320	1	2

3.3. Optimal extraction conditions

In this work, the extraction efficiency was represented by the total contents of the nine major ginsenosides calculated from the HPLC data. Of the nine, relatively polar saponins such as ginsenoside Rb1, Rb2, and Rc were dominant in both raw and heat-processed ginseng. Thus, the extraction efficiency was likely to be principally determined by the effects of the solvents on the extraction of the three major ginsenosides.

The optimal solvent for the extraction of polar ginsenosides Rb1 and Rg1 was 60% methanol. For both raw and heat-processed ginseng (major ginsenosides: Rb1, Rg1 and Rg3), the optimal solvent was 70% methanol in water.

Even though ginsenoside Rb1, Rb2, and Rc are dominant, heat-processed ginseng contains a substantial amount of artifactual saponins including ginsenoside (20*S*)-Rg3, Rg5, and Rk1, which are less polar than naturally-occurring saponins.

Intriguingly, ginsenoside Rg5 and Rk1 were found to be best extracted with 80% methanol.

3.4. LC group analysis of nine major ginsenosides in ginseng products

Up to now, most reports concerning quality control of ginseng products have been published [39, 40]. But for the carrying analysis of ginseng products by mentioned above reports [39, 40] a number of many ginsenosides are required. It's generally known that purchasing or isolation of ginsenosides is costly and time−consuming process.

The purpose of the study was to determine a simplified and inexpensivequality control method of ginseng and its products by using reference ginsenosides only.

Firstly all nine ginsenosides were sub categorized into the three groups with their reference compounds: where A is protopanaxatriol group (PPT), B is protopanaxadiol group (PPD) and less polar ginsenosides group is C (Table 5).

To calculate of total contents of each group ginsenosides Rg1, Rb1 and Rg3 standards were chosen as reference compounds, respectively. To obtain the total content of each group ginsenosides two factors, molecular weight of reference compound and total sum of molar concentrations of ginsenosides were used.

Results showed how the official KP method properly works on an example of white ginseng where Rg1 should be not less than 0.1mg and the content of Rb1 not less 0.2mg. When proposed method was applied for the analysis of ginsenosides in White ginseng, the composition of ginsenosides was sufficient. The content of ginsenosides Rg1 and Rb1 were 0.37mgand 1.41mg, respectively. It shown that the total content of protopanaxatriol and protopanxadiol group 1.23 mg and 3.91mg, respectively was good enough to validate the content of White ginseng. Application of official method for the analysis of heat-processed ginseng does not express the quality of product, because the content of Rg1 was degraded during steaming process.

On the contrary, application of the proposed method's results showed good enough total content of each group ginsenosides. The (A) protopanaxatriol group 0.38 mg, the (B) protopanaxadiol group 3.16 mg and (C) less polar ginsenosides group 1.90 mg, respectively.

According to the results of this experiment we can see the effectiveness of the proposed method for the analysis of the quality control of all kinds of ginseng products

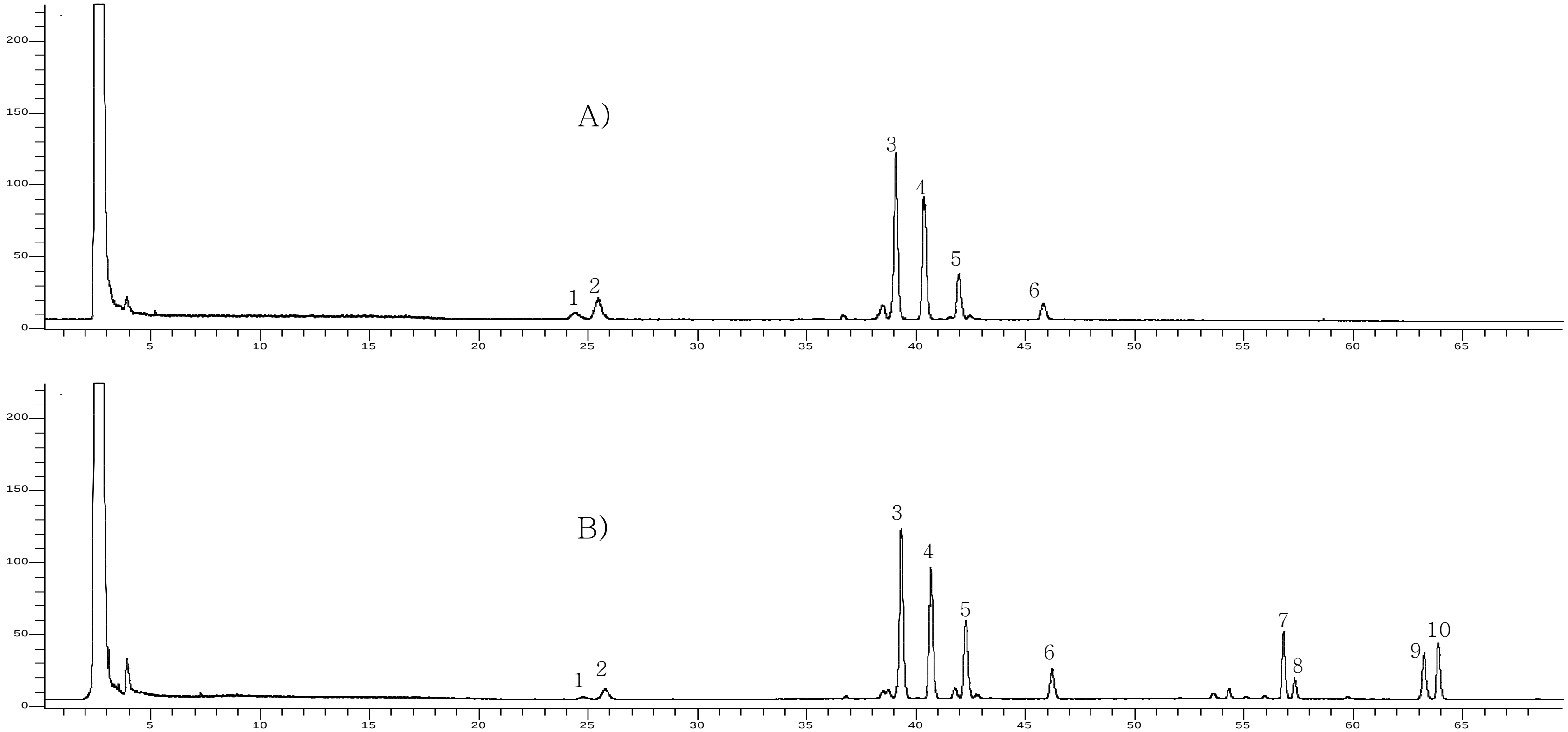


Figure 13. Representative LC-UV chromatograms of raw ginseng and heat-processed ginseng extracted with 70% aqueous methanol. (a) Raw ginseng (b) heat-processed ginseng. 1, Re; 2. Rg1; 3, Rb1; 4, Rc; 5, Rb2; 6,Rd; 7, Rg3(S); 8, (R); 9, Rk1; 10, Rg5

Table 5. Reference compounds of group ginsenosides

Group	Ginsenosides	Note	Reference compound
(A)	Re, Rg1	Polar PPTs	Rg1
(B)	Rb1, Rb2, Rc, Rd	Polar PPDs	Rb1
(C)	Rg3(*R*,*S*), Rg5, Rk1	Less polar artifacts	Rg3

Protopanaxatriol (PPT) type
Protopanaxadiol (PPD) type

4.1. Identification of ginsenosides in wild *Panaxvietnamensis.*

A total of 17 ginsenosides were detected by LC-ELSD (Figure 14). Due to the identical gradient conditions (column size, flow rate, and system capacity) in LC-Q-TOF-MS, retention times were similar to LC-ELSD, as confirmed using ginsenoside standards. A total of 11 ginsenosides were identified using standards: notoginsenoside R1, majonoside R1, ginsenoside Rg1, ginsenoside Re, majonoside R2, vina-ginsenoside R2, notoginsenoside R2, ginsenoside Rb1, ginsenosideRc, ginsenoside Rb2, and ginsenoside Rd. To identify six other unidentified metabolites, m/z of [M-H] - and fragment ions were measured by LC-Q-TOF-MS and compared to literature values [43-46]. Two ginsenosides (quinquenoside R1 and gypenoside XVII) were tentatively identified. The m/z value of the aglycone (459.4; protopanaxadiol) supported literature-based identification (Table 6). Only four metabolites detected by ELSD remained unidentified.

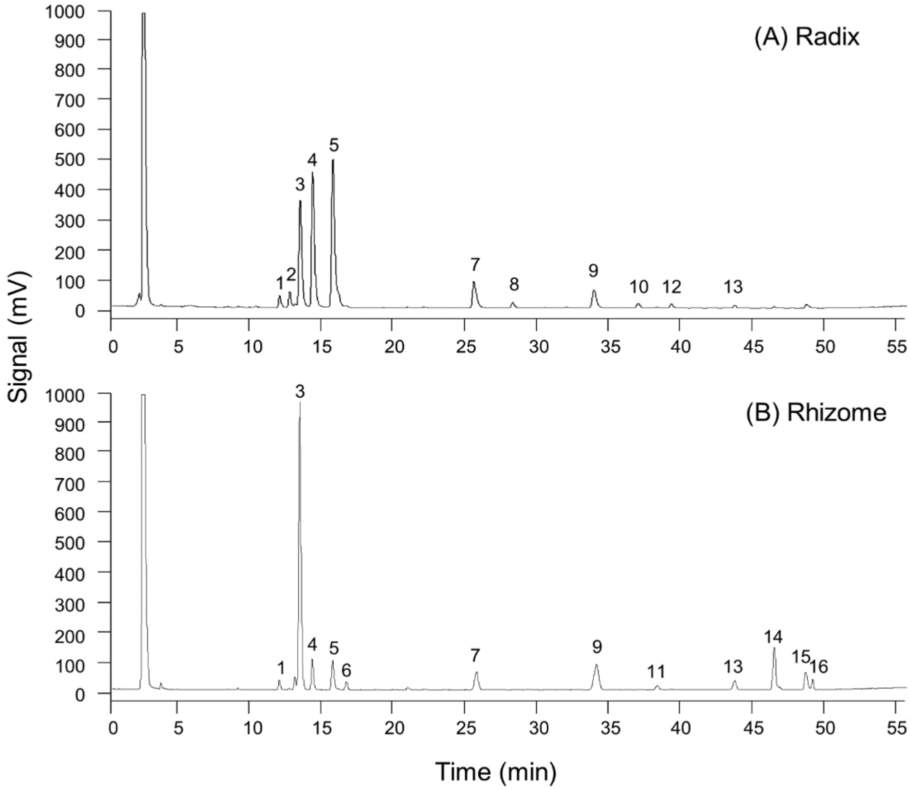


Figure 14.Representative ELSD chromatograms of wild *Panaxvietnamensis*. 1, Unidentified 1; 2,Notoginsenoside R1; 3, Majonoside R1; 4, Ginsenoside Rg1 and Ginsenoside Re; 5, Majonoside R2; 6, Unidentified 2; 7, Vina-ginsenoside R2; 8, Notoginsenoside R2; 9, Ginsenoside Rb1; 10, GinsenosideRc; 11, Unidentified 3 (Quinquenoside R1); 12, Ginsenoside Rb2; 13, Ginsenoside Rd; 14, Unidentified 4 (Gypenoside XVII); 15, Unidentified 5; 16, Unidentified 6

Table 6. Identification of ginsenosides in wild P. vietnamensis by LC−Q−TOF−MS

No.	Retention time (min)[a]	Measured m/z ([M−H]$^-$)		Exact m/z ([M−H]$^-$)	Formula	Identification	Part
		Parent	Fragment[b]				
1	12.1	961.5	799.5 637.4 475.4 (PPT[e])			Unidentified_1	Radix Rhizome
2	12.8	931.5		931.5	$C_{47}H_{80}O_{18}$	Notoginsenoside R1[C]	Radix
3	13.5	815.4		815.4	$C_{42}H_{72}O_{15}$	Majoniside R1[c]	Radix Rhizome
4	14.4	7899.4 945.5		799.4 945.4	$C_{42}H_{72}O_{14}$ $C_{48}H_{82}O_{18}$	Ginsenoside Rg1[c] +Ginsenoside Re[c]	Radix Rhizome
5	15.8	785.4		785.4	$C_{41}H_{70}O_{14}$	Majonoside R2[c]	Radix Rhizome
6	16.8	799.5	637.4 475.4 (PPT[e])			Unidentified_2	Rhizome
7	25.6	827.4		827.4	$C_{43}H_{72}O_{15}$	Vina−ginsenoside R2[c]	Radix Rhizome

8	28.4	769.4		769.4	$C_{41}H_{70}O_{13}$	Notoginsenoside R2[c]	Radix
9	34.1	1107.5		1107.5	$C_{54}H_{92}O_{23}$	Ginsenoside Rb1[c]	Radix Rhizome
10	37.1	1077.5		1077.5	$C_{53}H_{90}O_{22}$	GinsenosideRc[c]	Radix Rhizome
11	38.5	1149.6	1107.7 987.6 945.6 783.5	1149.6	$C_{56}H_{94}O_{24}$	Unidentified_3 (Quinquenoside R1[d])	Rhizome
12	39.4	1077.5		1077.5	$C_{53}H_{90}O_{22}$	Ginsenoside Rb2c	Radix
13	43.9	945.5		945.5	$C_{48}H_{82}O_{18}$	GinsenosideRd[c]	Radix
14	46.6	945.6	783.5 621.4 459.4 (PPD[f])	945.5	$C_{48}H_{82}O_{18}$	Unidentified_4 (GypenosideXVII[d])	Rhizome Rhizome
15	48.8	N.C. [g] N.C. [g]				Unidentified_5 Unidentified_6	Rhizome
							Rhizome

[a] Matched with LC−ELSD chromatogram
[b] For tentative identification, unidentified metabolites were analyzed by MS/MS
[c] Identified against standard

[d] Identified by comparison with literature values
[e] Measured m/z of protopanaxatriol (PPT)
[f] Measured m/z of protopanaxadiol (PPD)
[g] No connection between LC−ELSD chromatogram and LC−QToF−MS chromatogram

4.2. Quantification of ginsenosides in wild *Panaxvietnamensis*

Ocotillol type ginsenosides, commonly contained in *P. vietnamensis* varieties, are unsuited to UV detection due to the absence of chromophores (Figure 1), so an ELSD detector was used for quantification. Seventeen ginsenosides were divided into two groups in order of elution. Ginsenoside Rg1 and Rb1 were chosen as representative standards for the indirect quantification of the two groups. Linearity was evaluated by preparing six calibration points ranging from 15 to 500 μg/mL; R2 values for ginsenosides Rg1 and Rb1 were 0.9977 and 0.9991, respectively. Thus, both calibration curves exhibited linearity in the tested range.

The ginsenoside contents of *Panax vietnamensis* radix and rhizome are shown in Table 7. When unidentified ginsenosides were not considered, notoginsenoside R1, notoginsenoside R2, ginsenosideRc, and ginsenoside Rb2 were not detected in the rhizome, unlike the radix. Total content of identified ginsenosides in the radix and rhizome were 111.06 and 84.44 mg/g, respectively. The radix contained approximately 31 percent more ginsenoside than the rhizome, mainly due to differences in the contents of protopanaxatriol type ginsenosides: notoginsenoside R1, notoginsenoside R2, ginsenoside Rg1, and ginsenoside Re. Differences in the content of protopanaxadiol and ocotillol type ginsenosides were smaller than for the protopanaxatriol type. Moreover, ocotillol type ginsenosides accounted for the highest proportion of ginsenosides in wild *Panax vietnamensis.*

Table 7. Ginsenosides content of wild *Panax* vietnamensis (n=3)

No.	Compound	Content	
		Radix	Rhizome
1	Unidentified_1	2.25±0.39	3.48±1.46
2	Notoginsenoside R1[a]	5.30±2.92	N.D. [e]
3	Majoniside R1[c]	20.66±2.49	41.10±6.09
4	Ginsenoside Rg1[a] +Ginsenoside Re[a]	24.07±5.20	9.04±2.80
5	Majoniside R2[c]	32.44±10.72	8.21±3.02
6	Unidentified_2	N.D.	1.74±0.46
7	Vina-ginsenoside R2[c]	7.83±1.57	5.84±1.05
8	Notoginsenoside R2[a]	4.01±1.07	N.D.
9	Ginsenoside Rb1[b]	10.08±5.26	15.49±3.48
10	GinsenosideRc[b]	3.31±0.68	N.D.
11	Unidentified_3 (Quinquenoside R1)	N.D.	3.47±0.60
12	Ginsenoside Rb2[b]	2.01±0.50	N.D.
13	GinsenosideRd[b]	1.35±0.53	4.77±1.62
14	Unidentified_4 (Gypenoside XVII)	N.D.	7.10±4.78
15	Unidentified_5	N.D.	6.01±1.25
16	Unidentified_6	N.D.	2.28±1.27
PPT type		33.38±5.03	9.04±2.80
PPD type		16.75±5.27	20.25±4.86
Ocotillol type		60.93±13.07	55.15±4.26
Total ginsenoside		111.06±22.89	84.44±3.71

[a] Protopanaxadiol (PPT) type
[b]Protopanaxadiol (PPD) type
[c] Ocotillol type
[d]Calculated as the sum, except for unidentified ginsenosides
[e] Not detected

4.3. Comparison of ginsenoside content in radix and rhizome of three wild *Panax* species

Interestingly, dissimilar peak patterns were observed in the radix and rhizome of wild *Panax vietnamensis* (Figure 14). Because cultivated ginseng usually does not show this difference, we propose that this results from the distinctive long rhizome of wild ginseng [47-48]. Therefore, two parts of wild *Panax ginseng* and *Panax quinquefolius*, which also possess long rhizomes, were analyzed to determine whether this pattern occurs in other wild *Panax* ginseng species. Chromatograms of wild *P. ginseng* and *P. quinquefolius*are shown in Figures 15 and 16, and the quantitative results are shown in Tables 8 and 9. The variety and total content of ginsenosides in wild *P. ginseng* and *Panax quinquefolius* were much smaller than in wild *Panax vietnamensis.* Additionally, ocotillol type ginsenosides such as majonoside and vinaginsenoside were found exclusively in wild *Panax vietnamensis*, as mentioned above. Differences between the three wild

Panax species were summarized to verify our assumption about the differences between the parts (Figure 17). The difference was not observed in wild *P. ginseng* or *Panax quinquefolius*, unlike *Panax vietnamensis*. For confirmation of dissimilarity by statistical analysis, the Pearson's correlation coefficients [49]for the three species were calculated. Based on the results in Figure 17, the coefficient between radix and rhizome of wild *Panax vietnamensis* was 0.033 (weak correlation), whereas the coefficients of wild *Panax ginseng* and *Panax quinquefolius* were 0.958 and 0.998, respectively, indicating substantial positive correlations to wild *Panax vietnamensis*. In brief, the difference in ginsenoside composition between radix and rhizome is limited to *Panax vietnamensis*.

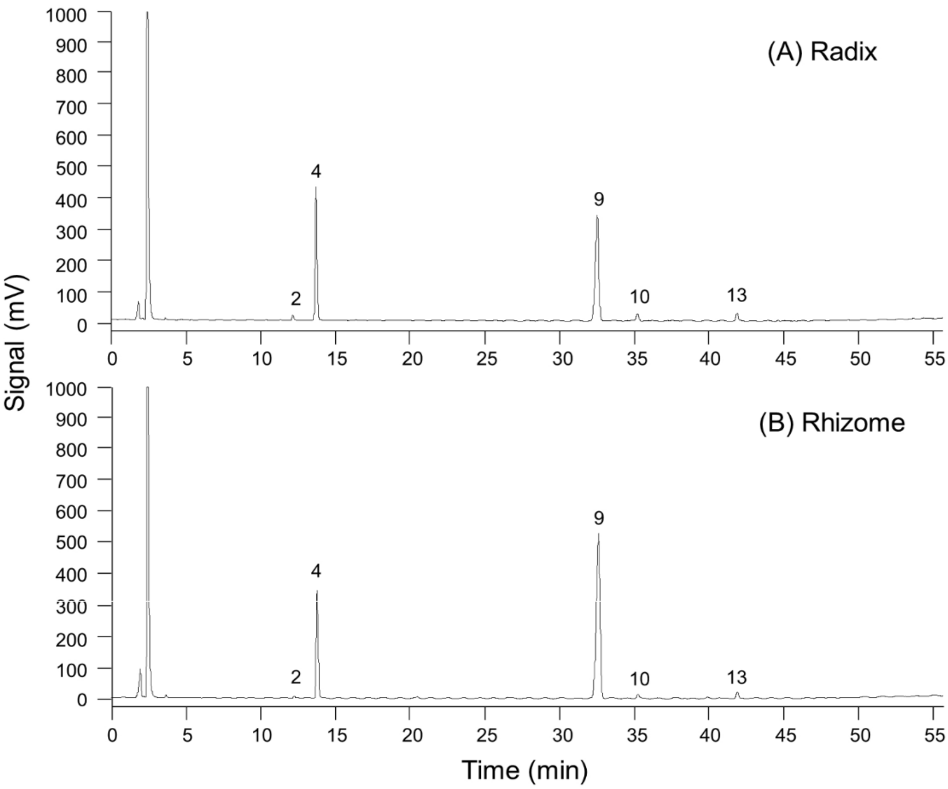


Figure 15.Representative ELSD chromatograms of wild *Panax ginseng.* 2, Notoginsenoside R1; 4,Ginsenoside Rg1 and Ginsenoside Re; 9, Ginsenoside Rb1; 10, Ginsenoside Rc; 12, Ginsenoside Rb2; 13, Ginsenoside Rd

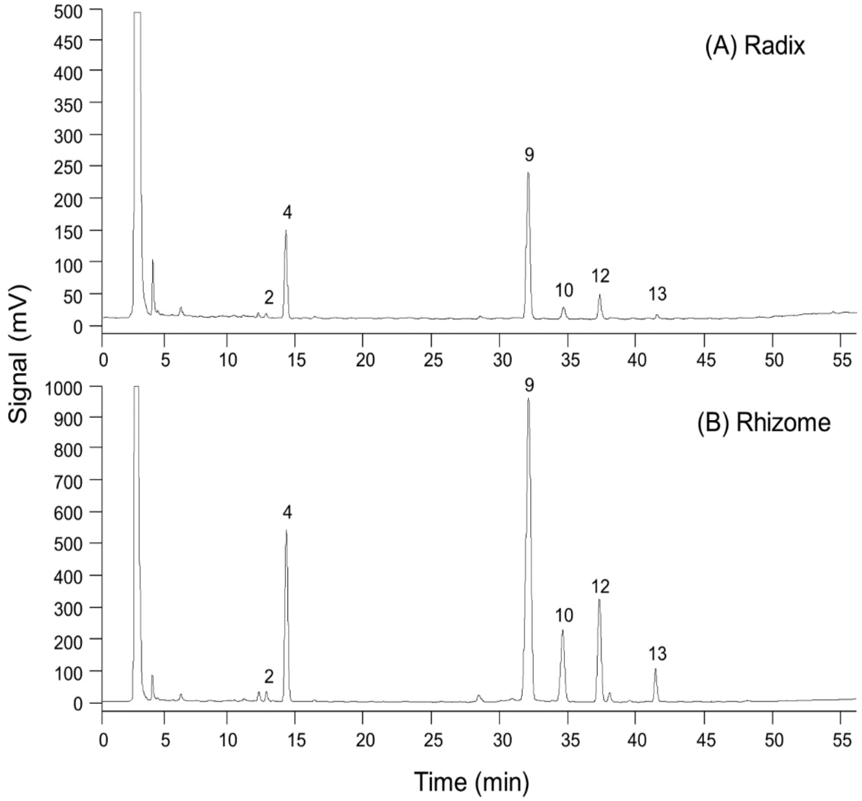


Figure 16.Representative ELSD chromatograms of wild *Panax* quinquefolius. 2, Notoginsenoside R1; 4, Ginsenoside Rg1 and Ginsenoside Re; 9, Ginsenoside Rb1; 10, Ginsenoside Rc; 13, Ginsenoside Rd

Table 8. Ginsenosides content of wild *Panax* ginseng (n=3)

No. [a]	Compound	Content mg/g	
		Radix	Rhizome
2	Notoginsenoside R1	0.27±0.03	0.83±0.17
4	Ginsenoside Rg1 +Ginsenoside Re	2.56±1.24	7.14±1.17
9	Ginsenoside Rb1	4.91±2.47	17.67±4.77
10	GinsenosideRc	1.35±1.12	5.03±2.06
12	Ginsenoside Rb2	0.82±0.53	5.55±2.73
13	Ginsenoside Rd	0.26±0.09	2.17±0.87
	Total ginsenosides	10.17±4.77	38.39±11.09

[a]Matched with the LC−ELSD chromatogram of *Panax vietnamensis*

Table 9. Ginsenosides content of wild *Panaxquinquefolius* (n=4)

No [a]	Compound	Content mg/g	
		Radix	Rhizome
2	Notoginsenoside R1	0.34±0.07	0.24±0.14
4	Ginsenoside Rg1 +Ginsenoside Re	3.85±1.18	4.13±0.70
9	Ginsenoside Rb1	7.49±3.90	9.39±5.09
10	GinsenosideRc	0.75±0.42	0.51±0.41
13	Ginsenoside Rd	0.82±0.64	0.66±0.53
	Total ginsenosides	13.25±6.13	14.93±6.13

[a] Matched with the LC−ELSD chromatogram of *Panaxvietnamensis*

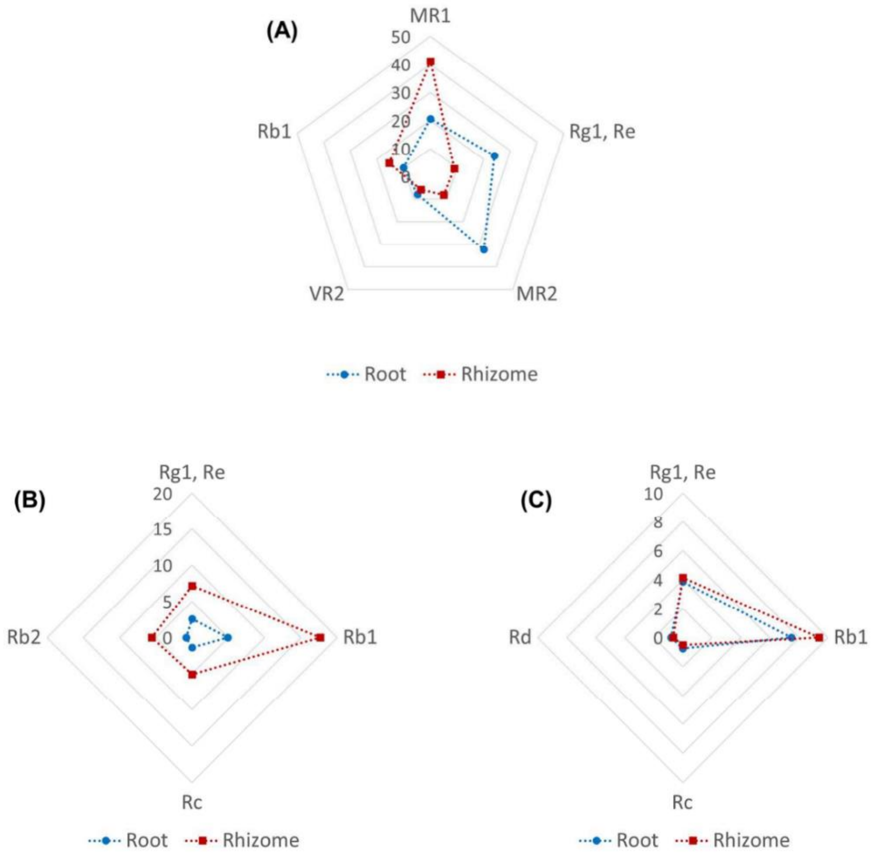


Figure 17. Comparative radar charts of ginsenosides in three different species of wild ginseng. Abundant ginsenosides in the radix and rhizome of each species were selected for pattern comparisons. (A) *P. vietnamensis;* (B) *P. ginseng;* (C) *P. quinquefolius*. Blue, Root; Red, Rhizome.

5. Conclusion

In this experiment, a new simplified and inexpensive analytical method was developed, which can be suitable forall kind of ginseng and its products.

A comparative analysis was conducted between official method (KP) and our proposed method. For quantitative analysis of each group ginsenosides reference compounds were chosen.

The results of quantitative analysis of each groups howed satisfactory enough content of ginsenosides in both raw and heat-treated ginseng samples, which confirms the effectiveness of our proposed method.

Two ginseng preparations, raw and heat-processed ginseng, have been quantitatively analyzed under different extraction conditions. Apparently, the quantification results were dependent on the solvent(s) for extraction. When the total contents of nine major ginsenosides were evaluated, the optimal conditions for the extraction of polar ginsenosides Rb1 and Rg1 were found to be approximately 60% methanol in water. For both raw and heat-processed ginseng (major ginsenosides: Rb1, Rg1 and Rg3), the optimal solvent was 70% aqueous methanol. However, 80% methanol in water was the best of the five tested solvent systems for the efficient extraction of ginsenoside Rg5 and Rk1. The results imply that a less polar solvent system might be better for the efficient extraction of a ginseng preparation containing a large amount of ginsenoside Rg5 and Rk1. We believe that our work will help raise the necessity of amending some regulations on ginseng products.

LC-ELSD and Q-TOF-MS/MS detected a total of 17 compounds in wild *Panax* vietnamensis.

We determined the concentrations of 17 compounds, 13 of which were identified. In Vietnamese ginseng, the major ginsenosides were of the ocotillol type. Furthermore, the difference in total ginsenoside content between radix and rhizome of wild *Panax vietnamensis* was 31%. This was caused by differences in protopanaxatriol type ginsenosides. We also confirmed that distinct differences in peak patterns between radix and rhizome occur only in wild *Panax vietnamensis*, even though all wild ginseng species have similar long rhizomes with a round radix. This study could be used as a reference for future study of the characteristics of *Panax vietnamensis.*

6. References

1. New World Encyclopedia http://www.newworldencyclopedia.org/entry/Ginseng
2. Chen, J.K.; Chen T.T., Chinese Medical Herbology and Pharmacology 2004
3. Huang, K.C., The Pharmacology of Chinese Herbs. CRC Press, Boca Raton, FL, 1999.
4. Ernst, E., *Panax* ginseng: an overview of the clinical evidenc, *J. of Ginseng Res* 2010 34(4), 259–263.
5. Choi K. T., Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax* ginseng C.A. Meyer, *Acta Pharmacologica Sinica* 2008 29(9), 1109–1118.
6. Qi L. W.; Wang C. Z.; Yuan C. S., Isolation and analysis of ginseng: advances and challenges, *Natural Product Reports* 2011, 28(3) 467–495.
7. Park J. D.; Rhee D. K.; Lee Y. H., Biological activities and chemistry of saponins from Panax ginseng C. A. Meyer, *Phytochemistry Reviews* 2005, 4(2–3), 159–175.
8. Karmazyn, M.; Moey, M.; Gan, X.T., Therapeutic potential of ginseng in the management of cardiovascular disorders, *Drugs* 2011 71(15) 1989–2008.
9. Buettner, C.; Yeh, G. Y.; Phillips, R. S.; Mittleman, M. A.; Kaptchuk, , Systematic review of the of ginseng on cardiovascular risk factors, *Annals of Pharmacotherapy* 2006, 40(1), 83–95.
10. Radad, K.; Gille, G.; Liu, L.; Rausch, W. D., Use of ginseng in medicine with emphasis on neurodegenerative disorders, *J of Pharmacological Sciences* 2006, 100(3), 175–186.
11. Lee, D. C. W.; Lau, A. S. Y., Effects of *Panax* ginseng on tumor necrosis factor–α–mediated inflammation: a mini–review,

Molecules 2011, 16(44), 2802-2816.

12. Attele, A. S.; Wu, J. A.; Yuan, C. S., Ginseng pharmacology: multiple constituents and multiple actions. *Biochemical Pharmacology* 1999, 58(11), 1685-1693.

13. Park, J.K.; Nam K.Y.; Hyun H.C.; Jin S.H.; Chepunov S.A; Chepunov N.E., Effect of ginseng triol saponin fractions on the spatial memory functions studied with 12-arm radial maze, *Korean J Ginseng Sci* 1994, 18: 32-8.

14. Saito, H.; Nishiyama, N., Effects of ginseng and its saponins on experimental amnesis in mice and on cell cultures of neurons, *Proceedings of the 5th International Ginseng Symposium* 1988.

15. Zhang, J.T.; Qu, Z.W.; Liu, Y.; Deng, H.L., Preliminary study on antiamnestic mechanism of ginsenoside Rg1 and Rb1, *Clin Med J* 1990; 103: 932-8.

16. Zhang, G.; Liu, A.; Zhou, Y.; San, X.; Jin, T.; Jin, Y., Ginsenoside- Rg2 protects memory impairment via anti-apoptosis in a rat model with vascular dementia, *J Ethnopharmacol* 2008; 115: 441-8. 9.

17. Lee, S.T.; Chu, K.; Kim, J.M.; Park, H.J.; Kim, M., Cognitive improvement by ginseng in Alzheimer' disease, *Proceedings of the 9th International Ginseng Symposium* 2006,

18. Seo, Y.J.; Shim E.J.; Kwon M.S; Choi O.S; Yang J.W.; Choi S.S., Anticiceptive effects of ginsenosides injected intracerebroventricularly or intrathecally in various pain models, *Proceedings of the 9th International Ginseng Symposium* 2006

19. Kamangar, F.; Gao, Y.T.; Shu, X.O.; Kahkeshani, K.; Ji, B.T.; Yang, G., Ginseng intake and gastric cancer risk in the Shanghai Women's Health Study cohort, *Cancer Epidemiol Biomarkers Prev* 2007, 16: 629-30.

20. Yun, T.K., Experimental and epidemiologic evidence on non-organ specific cancer preventive effect of Korean red ginseng and identification of active compounds, *Mutat Res* 2003, 523-524: 63-74,

21. Yun, T.K.; Choi, S.Y., A case-control study of ginseng intake and cancer. *Int J Epidemiol* 1990, 19: 871-6.

22. Yun, T.K; Choi, S.Y., Preventive effect of ginseng intake against various human cancers: A case-control study on 1987 pairs, *Cancer Epidemiol Biomakers Prev* 1995, 4: 401-8

23. Ahn, Y.K.; Kim, J.Y.; Chung, J.G.; Kim, J.H.; Goo, J.D., The effect of Korean ginseng on the immunotoxicity of mitomycin *C. Yakhak Hoeji* 1987, 31: 355-60.

24. Suh, S.O.; Kim. J.; Boo, Y.J.; Park, J.M.; Kim. J., Prospective study for Korean red ginseng extract as an immune modulator following a curative surgery in patients with advanced colon cancer, *Proceedings of the 9th International Ginseng Symposium* 2006.

25. Lai, D.M.; Tu, Y.K.; Liu, I.M.; Chen, P.F.; Cheng, J.T., Mediation of betaendorphin by ginsenoside Rh2 to lower plasma glucose in streptozotocin-induced diabetic rats, *Planta Med* 2006, 72: 9-13.

26. Sievenpiper, J.L.; Sung. M.K.; Di Buono M; Kim, S. K.; Nam, K.Y.; Arnason, J.T., Korean ginseng rootlet decreases acute postprandial glycemia: Results from sequential preparation-

and dose-finding studies. *J Am Coll Nutr* 2006, 25: 100-7.

27. Vuksan, V.; Sievenpiper, J.L.; Xu, Z.; Beljan-Zdravkovic, U.; Jenkins, A.L.; Arnason, J.T., A role for ginseng in the control of postprandial glycemia and type 2 diabetes, *Proceedings of the 8th International Symposium on Ginseng* 2002

28. Yokozawa, T.; Kobayashi, T.; Oura, H.; Kawashima, Y., Studies on the mechanism of hypoglycemic activity of ginsenoside-Rb2 streptozotocin-diabetic rats, *Chem Pharm Bull* 1985, 33: 869-72.

29. Yokozawa, T.; Oura, H.; Kawashima, Y., Effect of administration of ginsenoside-Rb2 in diabetic rats: In terms of carbohydrate and lipid metabolites, *Chem Pharm Bull* 1987, 35: 4872-7.

30. Yokozawa, T.; Oura, H., Facilitation of protein biosynthesis by ginsenoside-Rb2 administration in diabetic rats, *J Nat Prod* 1990, 53: 1514-18.

31. Ando, T.; Muraoka, T.; Okuda, H.; Yamasaki, N., Preparation of insulinlike peptides from Panax ginseng. *Proceedings of Wakan-Yaku Ginseng Symposium* 1979, 12: 15-19.

32. Lee, W.K.; Kao, S.T.; Liu, I.M.; Chen, J.T., Increase of insulin secretion by ginsenoside Rh2 to lower plasma glucose in Wistar rats. *Clin Exp Pharmacol Physiol* 2006, 33: 27-32.

33. Okuda, H.; Lee, S.D., Biological activities of non-saponincompounds isolated from Korean red ginseng. *Proceedings of the International Symposium on Korean Ginseng* 1990.

34. Takaku, T.; Kameda, K.; Matsuura, Y.;, Sekiya, K.;, Okuda, H.,

Studies on insulin-like substances of Korean red ginseng, *Planta Med* 1990, 56: 27-30.

35. Huh, K.; Jang, B.S.; Park. J.M.; Protective effect of ginseng on bromobenzene-induced hepatoxicity in mice. *Korean J Ginseng Sci* 1988 12: 114-20.

36. Lee, F.C.; Park, J.K.; Kim, E.K.; Ko, J.H.; Lee, J.S.; Kim, K.Y., The role of Panax ginseng in detoxification of xenobiotics. *Proceedings of the 4th International Ginseng Symposium* 1984.

37. Mizoguchi, Y., Protective effect of ginseng saponin. *Report of Ginseng Research Association* 1988, 46-48.

38. Saxena, P.N.; Mahour, K., Analysis of hepatoprotection by Panax ginseng following mercuric chloride intoxication in albino rat, *Proceedings of the 9th International Ginseng Symposium* 2006.

39. Song, J.H.; Park, M.J.; Kim, E.; Kim, Y.C., Effects of Panax ginseng on galactosamine-induced cytotoxicity in primary cultured rat hepatocytes, *Yakhak Hoeji* 1990, 34:341-7.

40. Matsuda, H.; Samukawa, K.; Kubo, M., Anti-hepatitic activity of ginsenoside Ro, *Planta Med* 1991, 57: 52-5.

41. Vuksan, V.; Stavro, M.; Woo, M.; Leiter, L.A.; Sung, M.K.; Sievenpiper, J.L., Korean red ginseng (Panax ginseng) can lower blood pressure in individuals with hypertension: a randomized controlled trial, *Proceedings of the 9th International Ginseng Symposium* 2006.

42. Kang, S.Y.; Schini-Kerth, V.B.; Kim, N.D., Ginsenosides of the protopanaxatriol group cause endothelium-dependent relaxation in the rat arorta, *Life Sci* 1995, 56: 1577-86.

43. Kim, N.D.; Kang, S.Y.; Schini-Kerth, V.B., Ginsenosides evoke endothelium-dependent vascular relaxation in rat aorta, *Gen Pharmac*1994, 25: 1071-7.

44. Saito, H.; Bao, T.T., Effect of red ginseng on mice exposed to various stress, *Proceedings of the 4th International Ginseng Symposium*1984

45. Kim, C.C., Influence of Panax ginseng on the response of stressful stimuli in the experimental animal exposed to various stress, *Korean J Ginseng Sci*1979, 3: 168-86.

46. Kaneko, H.; Nakanishi, K.; Murakami, A.; Aidoh, H.; Kuwashima, K., The acute effects of massive dose of red ginseng on healthy adults under the condition of cold stress, *Proceedings of the Korea-Japan Ginseng Symposium*1996.

47. Zhang, J.T.; Chui, D.H.; Liu, G.Z., The chemistry, metabolism and biological activities of ginseng, *Chemical Industry Press*2006.

48. El-Kady, A.A.; Hosain, A.R.M.; Park, M.H.; Kim, Y.T.; Park, H.H.; Abdel Wahhab, M.A., Efficacy of Panax ginseng extract standardized with ginsenoside Rg3 against oxidative stress of PCBs in rats, *Proceedings of the 9th International Ginseng Symposium* 2006.

49. Ogita, S.; Samugawa, K.; Clinical effectiveness of Korean ginseng on patients with climacteric disturbances, *Ginseng Rev*1994.

50. Andrade, E.; Mesquita, A.A.; Claro, J.A.; Andrade, P.M.; Ortiz, V; Paranhos, M.; Study of the efficacy of Korean red ginseng in the treatment of erectile dysfunction, *Asian J Androl*2007.

51. Choi, H.K.; Seong, D.H., Effectiveness for erectile dysfunction after the administration of Korean red ginseng, *Korean J Ginseng Sci*1995, 19: 17–21.

52. Choi, H.K.; Seong, D.H.; Rha, K.H., Evaluation of clinical efficacy of Korean red ginseng for erectile dysfunction by international index of erectile function (IIEF), *Int J Ginseng Res*2001, 25: 112–7.

53. Choi, Y.D.; Rha, K.H.; Choi, H.K., In vitro and in vivo experimental effect of Korean red ginseng on erection, *J Urol*1999, 162: 1508–11.

54. Salvati, G.; Genovesi, G.; Marcellini, L.; Paolini, P.; De Nuccio, I.;, Pepe, M., Effects of Panax ginseng C.A. Meyer saponins on male fertility *Panminerva Med*1996, 38: 249–54.

55. Cho, Y.K., Change of serum β2−microglobulin, p24 antigen and CD4+ T lymphocyte in persons with human immunodeficiency virus infection after azidothymidine Treatment, *J Korean Soc Microbiol*1993, 28: 409–17.

56. Cho, Y.K.; Kim, Y.K.; Lee, I.; Choi, M.H.; Shin, Y.O., The effect of Korean red ginseng (KRG), zidovudine, and the combination of KRG and ZDV on HIV−infected individuals, *J Korean Soc Microbiol*1996, 31: 353–60.

57. Shin, Y.O., Effects of Korea red ginseng on immunological markers of persons with human immunodeficiency virus, *Proceedings of the 6th International Ginseng Symposium*1993, 52–6.

58. Cho, Y.K.; Lim, J.Y.; Jung, Y.S.; Oh, S.K.; Lee, H.J.; Sung, H., High frequency of grossly deleted nef genes in HIV−1 infected long−term slow progressors treated with Korean red

ginseng, *Curr HIV Res* 2006 4: 447-57.

59. Abdel−Wahhab, M.A.; Ahmed, H.H., Protective effects of Korean Panax ginseng against chromium VI toxicity and free radical generation in rats, *J Ginseng Res*2004, 28: 11-7.

60. Chung, Y.H.; Kim, K.W.; Oura, H., Effects of ginsenoside Rb2 on the anti−oxidants in senescence−accelerated mice(SAM−R/1), *Proceedings of the 6th Internationa1 Ginseng Symposium*1993.

61. Han, B.H.; Han, Y.N.; Park, M.H., Chemical and biochemical studies on antioxidant components, *Advances in Chinese Medicinal Materials Research* 1985.

62. Kim, H.Y.; Chen, X.; Gillis, C.N., Ginsenosides protect pulmonary vascular endothelium against free radical−induced injury, *Biochem Biophys Res Commun*1992, 189: 670-6.

63. Kim, Y.K.; Gua, Q.; Packer, L., Free radical scavenging activity of red ginseng aqueous extracts, *Toxicology*2002,172: 149-56.

64. Kim, H.Y.; Lee, Y.H.; Kim, S.I., Effects of polyacetylene compounds from *Panax* ginseng C.A. Meyer on CCI4 induced lipid peroxidation in mouse liver, *Korean J Toxicol*1988, 4: 3.

65. Wang, X.M.; Qi, Y.; Sun, C.W.; Zhong, G.G.; Jiang, Y.; Qiu, Y.H., Single calcium channel analysis and electron spin resonance (ESR) spectral study on the mycocardial effects of ginsenoside Rb2, *Chung Guo Yao Tsa Chih*1994, 19: 621-4.

66. Oura, H.; Oita, Y., Effect of Korean red ginseng powder on the survival rate of rat, *Ginseng Rev*1989, 228-37.

67. Shibata, S.; Tanaka, O.; NagaI, M.; Ishit, T., Studies on the

constituents of Japanese and Chinese crude drugs. XII. Panaxadiol, a sapogenin of ginseng roots, *Chem Pharm Bull* 1963, 11:762-765

68. Shibata, S.; Ando, T.; Tanaka, O.; Meguro, Y.; Sôma, K.; Iida, Y., Saponins and sapogenins of *Panax ginseng* C.A. Meyer and some other *Panax* spp, *Yakugaku Zasshi* 1965, 85:753-755.

69. Shibata, S.; Tanaka, O.; Soma, K.; Aando,T.; Iida, Y.; Nakamura, H., Studies on saponins and sapogenins of ginseng. The structure of panaxatriol, *Tetrahedron Lett* 1965, 42:207-213.

70. Lu, J.M.; Yao, Q.; Chen, C., Ginseng compounds: an update on their molecular mechanisms and medical applications, *Curr Vasc Pharmacol* 2009, 7:293-302.

71. Nguyen, T.N., Study on *Panax* vietnamensis Ha et Grushv. Araliaceace. Botany, tissueculture-chemistry-biological properties, *Herbal Polonica* 1989, 35:1-229.

72. Duc, N.M.; Kasai, R.; Ohtani, K.; Ito, A.; Nguyen, T.N.; Yamasaki, K.; Tanaka, O., Saponins from Vietnamese ginseng, Panax vietnamensis Ha et Grushv. Collected in central Vietnam, *I Chem Pharm Bull* 1993, 41:2010-4.

73. Duc, N.M.; Kasai, R.; Ohtani, K.; Ito, A.; Nguyen, T.N.; Yamasaki, K.; Tanaka, O., Saponins from Vietnamese Ginseng, Panax vietnamensis Ha et Grushv. Collected in central Vietnam, *II. Chem Pharm Bull* 1994, 42:115-22.

74. Duc, N.M.; Kasai, R.; Ohtani, K.; Ito, A.; Nguyen, T.N.; Yamasaki, K.; Tanaka, O., Saponins from Vietnamese ginseng, Panax vietnamensis Ha et Grushv. collected in central Vietnam, *III. Chem Pharm Bull* 1994, 42:634-40.

75. Tran, L.Q.; Adnyana, I.; Tezuka, Y.; Nagaoka, T.; Tran, Q.; Kadota, S., Triterpene saponins from Vietnamese ginseng (*Panax* vietnamensis) and their hepatocytoprotective activity, *J Nat Prod*2001, 64:456-61.

76. Ovodov, Y. S.; Solov' eva, T. F., Polysaccharides of *Panax* ginseng. *Khimiya Prirodnykh Soedinenii*1966, 2, 299-303.

77. Matsunaga, H,; Katano, M.; Yamamoto,H.; Mori, M.; Takata, K., Studies on the panaxytriol of *Panax ginseng* C. A. Meyer. Isolation, determination and antitumor activity, *Chem Pharm Bull*1989, 37:1279-1281.

78. Iwabuchi, H.; Yoshikura, M.; Ikawa, Y.; Kamisako, W., Studies on the sesquiterpenoids of *Panax ginseng* C. A. Meyer. Isolation and structure determination of sesquiterpene alcohols, panasinsanols A and B, *Chem Pharm Bull* 1987, 35:1975-1981.

79. Iwabuchi, H.; Yoshikura, M.; Kamisako, W., Studies on the sesquiterpenoids of *Panax ginseng* C. A. Meyer. II. Isolation and structure determination of ginsenol, a novel sesquiterpene alcohol, *Chem Pharm Bull*1988, 36:2447-2451.

80. Iwabuchi, H.; Kato, N.; Yoshikura, M., Studies on the sesquiterpenoids of *Panax ginseng* C. A. Meyer, *IV. Chem Pharm Bull*1990, 38:1405-1407.

81. Wang, M.; Guilbert, L.J.; Li, J.; Wu, Y.; Pang, P.; Basu, T.K.; Shan, J.J., A proprietary extract from North American ginseng (*Panax quinquefolium*) enhances IL-2 and IFN-gamma productions in murine spleen cells induced by Con-A. *Int Immuno pharmacol*. 2004, 4:311-315.

82. Oshima, Y.; Sato, K.; Hikino, H., Isolation and hypoglycemic

activity of quinquefolans A, B, and C, glycans of *Panax quinquefolium* roots. *J Nat Prod* 1987, 50:188-190.

83. Christensen, L.P.; Jensen, M.; Kidmose, U., Simultaneous determination of ginsenosides and polyacetylenes in American ginseng root (*Panax quinquefolium* L.) by highperformance liquid chromatography. *J Agric Food Chem* 2006, 54:8995-9003.

84. Baranska, M.; Schulz, H.; Christensen, L.P., Structural changes of polyacetylenes in American ginseng root can be observed in situ by using Raman spectroscopy. *J Agric Food Chem*2006, 54:3629-3635.

85. Washida, D.; Kitanaka, S., Determination of polyacetylenes and ginsenosides in Panax species using high performance liquid chromatography. *Chem Pharm Bull*2003, 51:1314-1317.

86. Nakamura, S.; Sugimoto, S.; Matsuda, H.; Yoshikawa, M., Medicinal flowers. XVII. New dammarane-type triterpene glycosides from flower buds of American ginseng, *Panax quinquefolium* L. *Chem Pharm Bull* 2007, 55:1342-1348.

87. Ohtani, K.; Mizutani, K.; Hatono, S.; Kasai, R.; Sumino, R.; Shiota, T.; Ushijima, M.; Zhou, J.; Fuwa, T.; Tanaka, O., Sanchinan-A, a reticuloendothelial system activating arabinogalactan from sanchi-ginseng (roots of *Panax notoginseng*). *Planta Med*1987,53:166-169.

88. Choi, R.C.; Zhu, J.T.; Leung, K.W.; Chu, G.K.; Xie, H.Q.; Chen, V.P.; Zheng, K.Y.; Lau, D.T.; Dong, T.T.; Chow, P.C., A flavonol glycoside, isolated from roots of *Panax notoginseng*, reduces amyloid-beta-induced neurotoxicity in cultured neurons: signaling transduction and drugdevelopment for

Alzheimer' s disease. *J Alzheimers Dis* 2010, 19:795−811.

89. Wei, J.X.; Wang, L.A.; Du, H.; Li, R., Isolation and identification of sanchinoside B1 and B2 from rootlets of *Panax notoginseng* (Burk.) F. H. Chen. *Yao Xue Xue Bao* 1985, 20:288−293.

90. Liu, J.H.; Lee, C.S.; Leung, K.M.; Yan, Z.K.; Shen, B.H.; Zhao, Z.Z.; Jiang, Z.H., Quantification of two polyacetylenes in Radix Ginseng and roots of related *Panax* species using a gas chromatography−mass spectrometric method. *J Agric Food Chem* 2007, 55:8830−8835.

91. Chan, P.; Thomas, G.N.; Tomlinson, B., Protective effects of trilinolein extracted from *Panax notoginseng* against cardiovascular disease. *Acta Pharmacol Sin* 2002, 23:1157−1162.

92. Zhao, P.; Liu, Y.; Yang, C., Minor constituents from the roots of Panax notoginseng. *Acta Bot Yunnan* 1993, 15:409–412.

93. Lin, Q.; Zhao, X.; Liu, P.; Chen, Z.; Lu, Y., Studies on lipophilic constituents of Panax notoginseng. *Chin Tradit Herbal Drugs* 2002, 33:490–492.

94. Yoshikawa, M.; Murakami, T.; Ueno, T.; Hirokawa, N.; Yashiro, K.; Murakami, N.; Yamahara, J.; Matsuda, H.; Saijoh, R.; Tanaka, O.; Bioactive saponins and glycosides. IX. Notoginseng (2): structures of five new dammarane−type triterpene oligoglycosides, notoginsenosides−E, −G, −H, −I, and −J, and a novel acetylenic fatty acid glycoside, notoginsenic acid b−sophoroside, from the dried root of Panax notoginseng (Burk.) F. H. Chen. *Chem Pharm Bull* 1997, 45:1056– 1062.

95. Kobk−Larsen, M.; Christensen, L.P.; Vach, W.; Ritskes−Hoitinga, J.; Brandt, K., Inhibitory effects of feeding with

carrots or (−)−falcarinol on development of azoxymethane−induced preneoplastic lesions in the rat colon. *J Agric Food Chem* **2005,** 53:1823–1827.

96. Wei, J.X.; Wang, J.F.; Chang, L.Y.; Du, Y.C., Chemical studies of San−Chi Panax notoginseng (Burk.) F. H. Chen. I. Studies on the constituents of San−Chi root hairs. *Acta Pharm Sin* **1980**, 15:359–364.
97. Liu, R.; Zhang, J.; Wei, J.; Yu, H., Chemical constituents of Sanqi *(Panax notoginseng* kernel oil). Chin Tradit Herbal Drugs **1990** 21:242–245.

98. QuIlez, J.; GarcIa−Lorda, P.; Salas−Salvado, J., Potential uses and benefits of phytosterols in diet: present situation and future directions. *Clin Nutr* **2003,** 22:343–351.

99. Wei, J.X.; Wang, J.F., Studies on the flavonoids from the leaves of Panax notoginseng (Burk.) F. H. Chen. *Bull Chin Mater Med* **1987,** 12:671–673.
100. Heim, K.E.; Tagliaferro, A.R.; Bobilya, D.J., Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *J Nutr Biochem* **2002,** 13:572–584.
101. Hong, H.D.; Kim, Y.C.; Rho, J.H.; Kim, K.T.; Lee, Y.C., Changes on physicochemical properties of *Panax ginseng* C.A. Meyer during repeated steaming process. *J. Ginseng Res.* **2007**, 31: 222−229.
102. Kwak, Y.S.; Choi, K.H.; Kyung, J.S.; Won, J.Y.; Rhee, M.H.; Lee, J.G.; Hwang, M.S.; Lim, S.C.; Park, C.K.; Song, K.B.; Han, G.H., Effects high temperature heating on the physicochemical properties of Korean red ginseng (*Panax ginseng* C.A. Meyer) water extract. *J. Ginseng Res*. **2008,** 32:

120-126.

103. Kim, C.S.; Choi, K.J.; Kim, S.C.; Ko, S.Y.; Sung, H.S.; Lee, Y.G., Controls of the hydrolysis of ginseng saponins by neutralization of organic acids in red ginseng extract preparations. *J. Ginseng Res.* **1998,** 22: 205-210.

104. Kim, S.J.;, Murthy, H.N.; Hahn, E.J.; Lee, H.L.; Paek, K.Y., Effect of processing methods on the concentrations of bioactive components of ginseng (*Panax ginseng* C.A. Meyer) adventitious roots. *LWT Food Sci. Technol.* **2008**, 41: 959-964.

105. Lee, Y.J.; Kim, H.Y.; Kang, K.S.; Lee, J.G.; Yokozawa, T.; Park, J.H., The chemical and hydroxyl radical scavenging activity changes of ginsenoside-Rb1 by heat processing. *Bioorg. Med. Chem. Lett.* **2008**, 18:4515-4520.

106. Zhao, X.; Gao, L.; Wang, J.;, Bi, H.; Gao, J.; Du, X.; Zhou, Y.; Tai, G., A novel ginsenoside Rb1-hydrolyzing *β-D*-glucosidase from Cladosporium fulvum. *Process Biochem.* **2009,** 44: 612-618.

107. Cheng, L.Q.; Na, J.R.; Bang, M.H.; Kim, M.K.; Yang, D.C., Conversion of major ginsenoside Rb1 to 20(S)-ginsenoside Rg3 by Microbacterium sp. GS514. *Phytochemistry* **2008,** 69: 218-224.

108. Lee, Y.J.; Kim, H.Y.; Kang, K.S.; Lee, J.G.; Yokozawa T.; Park J.H., The chemical and hydroxyl radical scavenging activity changes of ginsenoside-Rb1 by heat processing. *Bioorg. Med. Chem. Lett.* **2008**, 18: 4515-4520.

109. Kim, Y.C.; Hong, H.D.; Rho, J.H.; Cho, C.W.; Rhee, Y.K.; Yim, J.H., Change of phenolic acid contents and radical

scavenging activities of ginseng according to steaming times. *J. Ginseng Res.*2007, 4: 230−236.

110. Yang, S.J.; Woo, K.S.; Yoo, J.S.; Kang, T.S.; Noh, Y.H.; Lee, J.S.; Jeong, H.S., Change of Korean ginseng components with high temperature and pressure treatment. *Korean J. Food Sci. Technol*. 2006, 38: 521−525.

111. Hwang, I.G.; Kim, Y.H.; Joung, E.M.; Woo, K.S.; Jeong, J.H.; Kwang W. Y.; Lee, J.S.; Jeong, H.S., Changes in Ginsenosides and Antioxidant Activity of Korean Ginseng (*Panax ginseng* C.A. Meyer) with Heating Temperature and Pressure *Food Sci. Biotechnol*. 2010, 19(4): 941−949.

112. Kitagawa, I.; Yoshikawa, M.; Yoshihara, M.; Hayashi, T.; Taniyama, T., Chemical studies on crude drug procession. I. On theconstituents of Ginseng Radix Rubra. *Yakugaku Zasshi*1983, 103: 612–622.

113. Kasai, R.; Besso, H.; Tanaka, O.; Saruwatari, Y.I.; Mizutare, T., Saponins of red ginseng. *Chem. Pharm. Bull.* 1983 31: 2120–2125.

114. Park, I.H,; Piao, L.Z.; Kwon, S.W.; Lee, Y.J.; Cho, S.Y.; Park, M.K.; Park, J.H., Cytotoxic dammarane glycosides from processed ginseng. *Chem Pharm Bull*. 2002, 50(4):538–540.

115. Kim, J.H.; Park, C.Y.; Lee, S.J., Effects of sun ginseng on subjective quality of life in cancer patients: a double−blind, placebo−controlled pilot trial. *J Clin Pharm Ther*. 2006, 31(4):331–334.

116. Choi, P.; Park, J. Y.; Kim, T.; Park, S. H.; Kim, H.; Kang, K. S.; Ham, J. Improved anticancer effect of ginseng extract by microwave−assisted processing through the 270

generation of ginsenosides Rg3, Rg5 and Rk1. *J. Funct. Foods* **2015**, *14*, 613-622.

117. Park, J. Y.; Choi, P.; Kim, T.; Ko, H.K.; Kim, H.K.; Kang, K. S.; Ham, J. Y.; Protective Effects of Processed Ginseng and its Active Ginsenosides on Cisplatin-Induced Nephrotoxicity: In Vitro and In Vivo Studies,*J. Agric. Food Chem* Publication Date (Web): 06 Jun **2015**.

118. Kang, K. S.; Yamabe, N.;Kim, H. Y.; Park, J.H.; Yokozawa, T.,Effects of Heat-Processed Ginseng and Its Active Component Ginsenoside 20(*S*)-Rg3 on the Progression of Renal Damage and Dysfunction in Type 2 Diabetic Otsuka Long-Evans Tokushima Fatty Rats, *Biol. Pharm. Bull.* ***2010,***33(6) 1077—1081.

119. Kim, K.T.; Yoo, K.M.; Lee J.W.; Eom, S.H.; Hwang, I. K.; Lee, C. Y., Protective effect of steamed American ginseng (*Panax quinquefolius* L.) on V79-4 cells induced by oxidative stress Journal of Ethnopharmacology 111 (2007) 443-450

120. Ren, G.X.; Chen, F., Degradation of ginsenosides in American ginseng (Panax quinquefolium) extracts during microwave and conventional heating. J Agr Food Chem. 1999; 47:1501-1505.

121. Wang, C.Z.; Aung, H.H.; Ni, M.; Wu, J.A.; Tong, R.B.; Wicks, S,; He, T.C.; Yuan, C.S., Red American ginseng: Ginsenoside constituents and antiproliferative activities of heat-processed *Panax quinquefolius* roots. *Planta Med*. **2007a**, 73:669-674.

122. Wang, C.Z.; Zhang, B.; Song, W.X.; Wang, A.B.; Ni, M.; Luo, X.J.; Aung, H.H.; Xie, J.T.; Tong, R.; He, T.C.; Yuan, C.S.,

Steamed American ginseng berry: Ginsenoside analyses and anticancer activities. *J Agr Food Chem*. **2006**, 54:9936–9942.

123. Xie, J.T.; Wang, C.Z.; Zhang, B.; Mehendale, S.R.; Li, X.L.; Sun, S.; Han, A.H.; Du, W.; He, T.C.; Yuan, C.S., In vitro and in vivo anticancer effects of American ginseng berry: Exploring representative compounds. *Biol Pharm Bull.***2009**, 32:1552–1558.

124. Wang, C.Z.; Li, X.L.; Wang, Q.F.; Mehendale, S.R.; Fishbein, A.B.; Han, A.H.; Sun, S.; Yuan, C.S., The mitochondrial pathway is involved in American ginseng-induced apoptosis of SW-480 colon cancer cells. *Oncol Rep*. **2009a**, 21:577–584.

125. Sun, S.; Qi, L.W.; Du, G.J.; Mehendale, S.R.; Wang, C.Z.; Yuan, C.S., Red notoginseng: higher ginsenoside content and stronger anticancer potential than Asian and American ginseng. *Food Chem*. **2011**, 125:1299–1305.

126. Lau, A.J.; Woo, S.O.; Koh, H.L., Analysis of saponins in raw and steamed Panax notoginseng using high-performance liquid chromatography with diode array detection. *J Chromatogr A***2003**, 1011:77-87.

127. Chan, E.C.; Yap, S.L.; Lau, A.J.; Leow, P.C.; Toh, D.F.; Koh, H.L., Ultra-performance liquid chromatography/time-of-flight mass spectrometry based metabolomics of raw and steamed *Panax notoginseng*. *Rapid Commun Mass Spectrom***2007**, 21:519-528.

128. Lau, A.J.; Seo, B.H.; Woo, S.O.; Koh, H.L., High-performance liquid chromatographic method with quantitative comparisons of whole chromatograms of raw and steamed

Panax notoginseng. *J Chromatogr A* 2004, 1057:141-149.

129. Toh, D.F.; New, L.S.; Koh, H.L.; Chan, E.C., Ultra-high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOFMS) for time-dependent profiling of raw and steamed Panax notoginseng. *J. Pharm Biomed Anal*2010, 52:43-50.

130. Sun, S.; Wang, C.Z.; Tong, R.; Li, X.; Fishbein, A.; Wang, Q.; He, T.; Du, W.; Yuan, C., Effects of steaming the root of Panax notoginseng on chemical composition and anticancer activities. *Food Chem*2010, 118:307-314.

131. Lau, A.J.; Toh, D.F.; Chua, T.K.; Pang, Y.K.; Woo, S.O.; Koh, H.L., Antiplatelet and anticoagulant effects of Panax notoginseng: comparison of raw and steamed Panax notoginseng with Panax ginseng and *Panax quinquefolium*. *J Ethnopharmacol*2009, 125:380-386.

132. Toh,D.F. Patel, D.N.; Chan, E.C.Y.; Teo,A,; Neo,S.Y.; Koh, H.L., Anti-proliferative effects of raw and steamed extracts of *Panax notoginseng* and its ginsenoside constituents on human liver cancer cells *Chinese Medicine*2011, 6:4.

133. Konoshima, T.; Takasaki, M.; Tokuda, H., Anti-carcinogenic activity of the roots of Panax notoginseng. *II. Biological and Pharmaceutical Bull*, 1999, 22(10), 1150-1152.

134. Wang, C. Z.; Xie, J. T.; Zhang, B.; Ni, M.; Fishbein, A.; Aung, H. H., Chemopreventive effects of Panax notoginseng and its major constituents on SW-480 human colorectal cancer cells. International *J of Oncology*2007, 31(5), 1149-1156.)

135. Sun, S.; Wang, C.Z.; Tong,R.; Li, X.L.; Fishbein, A.;

Wang, Q.; He, T.C.; Du , W.; Yuan, C.S., Effects of steaming the root of *Panax notoginseng* on chemical composition and anticancer activities *Food Chemistry* 2010, 118 307–314.

136. Le Van .T.H.; Lee S.Y.; Kim, T.R.; Kim, J.Y.; Kwon, S.W.; Nguyen, N.K.; Park, J.H.; Nguyen, M.D., Processed Vietnamese ginseng: preliminary results in chemistry and biological activity. *J Ginseng Res* 2014, 38:154–9.

137. Le Van, T.H.; Lee , S.Y.; Lee , G.J.; Nguyen , N.K.; Park, J.H.; Nguyen, M. D., Effects of steaming on saponin compositions and antiproliferative activity of *Vietnamese ginseng*. *J Ginseng Res* xxx (2015) 1–5.

138. Noh, K.H.; Son, J.W.; Kim, H.J.; Oh, D.K., Ginsenoside compound K production from ginseng root extract by a thermostable beta–glycosidase from Sulfolobus solfataricus. *Biosci Biotechnol Biochem* 2009, 73:316–21.

139. Ko, S.R.; Suzuki, Y.; Choi, K.J.; Kim, Y.H., Enzymatic preparation of genuine prosapogenin, 20(S)–ginsenoside Rh1, from ginsenosides Re and Rg1. *Biosci Biotechnol Biochem* 2000, 64:2739–43.

140. Mochizuki, M.; Yoo, Y.C.; Matsuzawa, K.; Sato, K.; Saiki, I.; Tonooka, S.; Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside–Rb2, 20(R)– and 20(S)–ginsenoside–Rg3, of red ginseng. *Biol Pharm Bull* 1995, 18:1197–202

141. Mochizuki, M.; Yoo, Y.C.; Matsuzawa, K.; Sato, K.; Saiki, I.; Tonooka, S.; Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside–Rb2, 20(R)– and 20(S)–ginsenoside–Rg3, of red ginseng. *Biol Pharm Bull* 1995, 18:1197–202.

142. Bae, E. A.; Han, M. J.; Choo, M. K.; Park, S. Y.; Kim,

D. H., *Bio & Phar Bull*2002, 25, 58-63.;

143. Bae, E. A.; Han, M. J.; Kim, E. J.; Kim, D. H., *Arch of Pharmacal Res*2004, 27, 61-67.

144. Trinh, H.T.; Han, S. J.; Kim, S. W.; Lee, Y. C.; Kim, D. H., J of Microb and Biotech 2007, 17, 1127-1133.

145. Cho, H. J.; Jung, E. Y.; Oh, S. H.; Yoon, B.; Suh, H. J.; Lee, H. S., J of Food Sci & Nutr 2010, 15, 105-112.

146. Kim, B.G.; Shin, K.S.; Yoon, T.J.; Yu, K.W.; Ra, K.S.; Kim J.M.; Kim, S.Y.; Suh, H.J., Fermentation of Korean Red Ginseng by Lactobacillus plantarum M-2 and Its Immunological Activities. *Appl Biochem Biotechnol* 2011, 165, 1107-1119.

147. Kim, B.G.; Choi, S.Y.; Kim, M.R.; Suh, H.J.; Park, H.J., Changes of ginsenosides in Korean red ginseng (Panax ginseng) fermented by Lactobacillus plantarum M1, *Process Biochemistry*2010, 45, 1319-1324

148. Ramesh, T.; Kim, S.W.; Sung, J.H.; Hwang, S.Y.; Sohn , S.H.; Yoo, S.K.; Kim, S.K., Effect of fermented Panax ginseng extract (GINST) on oxidative stress and antioxidant activities in major organs of aged rats *Experimental Gerontology*2012 47, 77-84.

149. Bae, E.A.; Park, S.Y.; Kim, D.H.,Constitutive beta-glucosidases hydrolyzing ginsenoside Rb1 and Rb2 from human intestinal bacteria. *Biol & Pharm Bull*2000, 23, 1481-1485.

150. Bae, E.A.; Choo, M.K.; Park, E.K.; Park, S.Y.; Shin, H.Y.; Kim, D.H., Metabolism of ginsenoside R(c) by human intestinal bacteria and its related anti allergic activity. *Biol & Pharm Bull*2002, 25, 743-747.

151. Hasegawa, H., Proof of the mysterious efficacy of ginseng: basic and cli- nical trials: Metabolic activation of ginsenoside: Deglycosylation by intesti-al bacteria and esterification with fatty acid. *J of Pharm Sci* **2004**, 95, 153-157.

152. Zhang, D.; Wang, Y.; Han, J.; Yu, W.; Deng, L.; Fawcett, J.P.; Liu, Z.; Gu, J., Rapid and sensitive LC-MS/MS assay for the quantitation of 20(S)-proto-panaxadiol in human plasma. *J of Chrom B* **2009a** 877, 581-585.

153. Zhou, W.; Yan, Q.; Li, J.Y.; Zhang, X.C.; Zhou, P., Biotransformation of Panax notoginseng saponins into ginsenoside compound K production by Paecilomyces bainier sp. 229. J of Appl Microb **2008b** 104, 699-706.

154. Noh, K.H.; Son, J.W.; Kim, H.J.; Oh, D.K., Ginsenoside compound K production from ginseng root extract by a thermostable beta-glycosidase from Sulfolobus solfataricus . Biosci Biotechnology and Biochemistry **2009**, 73, 316-321.

155. Zhou, W.; Feng, M.Q.; Li, J.Y.; Zhou, P., Studies on the preparation, crystal structure and bioactivity of ginsenoside compound K. J of Asian Nat Prod Res **2006,** 8, 519-527.

156. Choi, K.; Choi, C., Proapoptotic ginsenosides compound K and Rh enhance Fas-induced cell death of human astrocytoma cells through distinct apoptotic signaling pathways. *Cancer Research and Treatment* **2009** 41, 36-44.

157. Fuzzati, N., Analysis methods of ginsenosides. *J Chromatogr B AnalytTechnol Biomed Life Sci.* **2004**, 812, 119-133.

158. Oleszek, W.A., Chromatographic determination of plant saponins. *J Chromatogr A.*2002, 967, 147-162.

159. Oleszek, W.; Bialy, Z., Chromatographic determination of plant saponins: an update (2002-2005). *J Chromatogr A.*2006, 1112, 78-91.

160. Lui, J.H.-C.; Staba, E.J.; J. Nat. Prod., 1980, 43, 340.

161. Corthout, J.; Naessens, J.; Apers, S.; Vlietinck, A.J., *J. Pharm. Biomed. Anal*1999, 21,187.

162. Kim, Y.K.; Yoo, D.S.; Xu, H.; Park, N.I.; Kim, H.H.; Choi, J.E.; Park, S.U., Ginsenoside content of berries and roots of three typical Korean ginseng (*Panax ginseng*) cultivars. *Nat Prod Commun*2009, 4, 903-906.

163. Soldati, F.; Sticher, O.; HPLC separation and quantitative determination of ginsenosides from *Panax ginseng*, *Panax quinquefolium* and from ginseng drug preparations. 2nd communication *Planta Med., 38*1980, 348

164. Pietta, P.; Mauri, P.; Rava, A., Improved high-performance liquid chromatographic method for the analysis of ginsenosides in Panax Ginseng extracts and products *J. Chromatogr*1986, 356, 212.

165. Yamaguchi, H.; Kasai, R.; Matsuura, H.; Tanaka, O.; Fuwa, T., High-performance liquid chromatographic analysis of acidic saponins of ginseng and related plants. *Chem. Pharm. Bull*1988, 36 3468.

166. Guldager Petersen, T.; Palmqvist, B.; *J. Chromatogr*1990, 504,139.

167. Guedon, D.; Abbe, P.; Cappelaere, N.; Rames, N.,

Determination of saponosides from Panax ginseng C.A. Meyer. Comparison between official methods and HPLC. *Ann. Pharmaceutiques Françaises*1989, 47, 169.

168. Kanazawa, H.; Nagata, Y.; Kurosaki, E.; Matsushima, Y.; Takai, N., Comparison of columns of chemically modified porous glass and silica in reversed-phase high-performance liquid chromatography of ginsenosides. *J. Chromatogr*1993, 632, 79.

169. Lang, W.; Lou, Z.; But, P.P., A new HPLC method for the analysis of ginsenosides in *Panax ginseng*. J. Chin. Pharm. Sci **1993,** 2, 133.

170. Gomez-Serranillos, M.P.; Palomino, O.M.; Carretero, E.; Villar, A.; Cases, M.A. A new HPLC method for the analysis of ginsenosides in *Panax ginseng*. *Fitoterapia*1997, 68 533.

171. Chuang, W.C.; Sheu, S.J., Determination of ginsenosides in ginseng crude extracts by high-performance liquid chromatography. J Chromatogr A **1995**, 685, 243.

172. Qi, L.W.; Wang, C.Z.; Yuan, C.S., Isolation and analysis of ginseng: advances and challenges. *Nat Prod Rep*2011, 28, 467-495.

173. Park, M.K.; Park, J.H.; Han, S.B.; Shin, Y.G.; Park, I.H., High performance liquid chromatographic analysis of ginseng saponins using evaporative light scattering detection. *J Chromatogr A*1996, 736, 77-81.

174. Kwon, S.W.; Han, S.B.; Park, I.H.; Kim, J.M.; Park, M.K.; Park, J.H., Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J Chromatogr A*2001,

921, 335−339.

175. Kim, S.N.; Ha, Y.W.; Shin, H.; Son, S.H.; Wu, S.J.; Kim, Y.S., Simultaneous quantification of 14 ginsenosides in *Panax ginseng* C.A. Meyer (Korean red ginseng) by HPLC−ELSD and its application to quality control. *J Pharm Biomed Anal* 2007, 45, 164−170.

176. Qu, C.; Bai, Y.; Jin, X.; Wang, Y.; Zhang, K.; You, J.; Zhang, H., Study on ginsenosides in different parts and ages of *Panax quinquefolius* L. *Food Chem* 2009, 115, 340−346.

177. Sun, B.S.; Gu, L.J.; Fang, Z.M.; Wang, C.Y.; Wang, Z.; Lee, M.R.; Li, Z.; Li, J.J.; Sung, C.K., Simultaneous quantifi cation of 19 ginsenosides in black ginseng developed from *Panax ginseng* by HPLC−ELSD. *J Pharm Biomed Anal* 2009, 50, 15− 22.

178. Wan, J.B.; Li, S.P.; Chen, J.M.; Wang, Y.T., Chemical characteristics of three medicinal plants of the *Panax* genus determined by HPLC−ELSD. *J Sep Sci* 2007, 30, 825−832.

179. Zhang, X.; Ma, X.; Si, B.; Zhao, Y., Simultaneous determination of five active hydrolysis ingredients from *Panax quinquefolium* L. by HPLC−ELSD. *Biomed Chromatogr* 2011, 25, 646−651.

180. Lee, D.Y.; Cho, J.G.; Lee, M.K.; Lee, J.W.; Lee, Y.H.; Yang, D.C.; Baek, N.I., Discrimination of *Panax ginseng* roots cultivated in different areas in Korea using HPLC−ELSD and principal component analysis. *J Ginseng Res* 2011, 35,31−38.

181. Bai, C.C.; Han, S.Y.; Chai, X.Y.; Jiang, Y.; Li, P.; Tu, P.F., Sensitive determination of saponins in *Radix et Rhizoma Notoginseng* by charged aerosol detector coupled with HPLC. *J Liq Chromatogr Relat Technol* 2009, 32,242−260.

182. Wang, L.; He, W.S.; Yan, H.X.; Jiang, Y.; Bi, K.S.; Tu, P.F., Performance evaluation of charged aerosol and evaporative light scattering detection for the determination of ginsenosides by LC. *Chromatographia* 2009, 70, 603-608.

183. Park, M.K.; Kim, B.K.; Park, J.H.; Shin, Y.G., High performance liquid chromatographic determination of ginsenosides using photoreduction fluorescence detection. *J. Liq. Chromatogr*1995, 18, 2077.

184. Park, M.K.; Kim, B.K., Park, J.H.; Shin, Y.G.; Cho, K.H.; Do, Y.M., Photoreactivity of anthraquinones for the analysis of ginsenosides using photoreduction fluorescence detection-HPLC. *Arch. Pharm. Res*1996, 19, 562.

185. Shangguan, D.; Han, H.; Zhao, R.; Zhao, Y.; Xiong, S.; Liu, G., New method for high-performance liquid chromatographic separation and fluorescence detection of ginsenosides. *J Chromatogr A*2001, 910, 367.

186. Park, M.K.; Park, J.H.; Lee, M.Y.; Kim, S.J.; Park, I.J., Microanalysis of ginseng saponins by ion chromatography with pulsed amperometric detection. *J Liq Chromatogr*1994, 17,1171-1181.

187. Joo, K.M.; Park, C.W.; Jeong, H.J.; Lee, S.J.; Chang, I.S., Simultaneous determination of two Amadori compounds in Korean red ginseng (*Panax ginseng*) extracts and rat plasma by high-performance anion-exchange chromatography with pulsed amperometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci*2008, 865, 159-166.

188. Kwon, H.J.; Jeong, J.S.; Lee, Y.M.; Hong, S.P., A

reversed-phase high-performance liquid chromatography method with pulsed amperometric detection for the determination of glycosides. *J Chromatogr A*2008, 1185, 251-257.

189. Kwon, H.J.; Jeong, J.S.; Sim, H.J.; Lee, Y.M.; Kim, Y.S.; Hong, S.P., Sensitive high-performance liquid chromatography method of non-polar ginsenosides by alkaline enhanced pulsed amperometric detection. *J Chromatogr A* 2009, 1216, 4445-4450.

190. Eugster, P.J.; Guillarme, D.; Rudaz, S.; Veuthey, J.L.; Carrupt, P.A; Wolfender, J.L., Ultra high pressure liquid chromatography for crude plant extract profi ling. *J AOAC Int*2011, 94,51-70.

191. Dan, M.; Su, M.; Gao, X.; Zhao, T.; Zhao, A.; Xie, G.; Qiu, Y.; Zhou, M.; Liu, Z.; Jia, W., Metabolite profiling of *Panax notoginseng* using UPLC-ESI-MS. *Phytochemistry*2008, 69, 2237-2244.

192. Xie, G.;, Plumb, R.; Su, M.; Xu, Z.; Zhao, A.; Qiu, M.; Long, X.; Liu, Z.; Jia W. Ultra-performance LC/TOF MS analysis of medicinal *Panax* herbs for metabolomic research. *J Sep Sci*2008, 31, 1015-1026.

193. Qiu, Y.; Lu, X.; Pang, T.; Ma, C.; Li, X.; Xu, G., Determination of radix ginseng volatile oils at different ages by comprehensive two-dimensional gas chromatography/time-offlight mass spectrometry. *J Sep Sci* 2008, 31, 3451-3457.

194. Abd El-Aty, A.M.; Kim, I.K.; Kim, M.R.; Lee, C.; Shim, J.H., Determination of volatile organic compounds generated

from fresh, white and red *Panax ginseng* (C. A. Meyer) using a direct sample injection technique. *Biomed Chromatogr*2008, 22, 556-562.

195. Liu, J.H.; Lee, C.S.; Leung, K.M.; Yan, Z.K.; Shen, B.H.; Zhao, Z.Z.; Jiang, Z.H., Quantification of two polyacetylenes in Radix ginseng and roots of related *Panax* species using a gas chromatography-mass spectrometric method. *J Agric Food Chem*2007, 55, 8830-8835.

196. Angelova, N.; Kong, H.W.; Heijden, R.; Yang, S.Y.; Choi, Y.H.; Kim, H.K.; Wang, M.; Hankemeier, T.; Greef, J.; Xu, G., Recent methodology in the phytochemical analysis of ginseng. *Phytochem Anal*2008, 19, 2- 16.

197. Jung, D. W.; Lee, J. M.; Sung, C. K. Enzyme-linked immuno-sorbent assay for the determination of 20(S)-protopanaxatriol.Anal. Chim. Acta 462, 157-163 (2002).

198. Osinski, J.; Yuan, C.-S. American ginseng leaf: ginsenosideanalysis and hypoglycemic activity.Pharmacol. Res.,49,113-117 (2004).

199. Shao, Z.-H.; Xie, J.-T.; Hoek, T. L. C.; Menhendale, S.; Aung,H.; Li, C.-Q.; Qin, Y.;Schumacker, P. T.; Becker, L. B.; Yuan,C.-S. Antioxidant effects of American ginseng berry extract incardiomyocytes exposed to acute oxidant stress.Biochim. Bio-phys. Acta ,1670, 165-171 (2004).

200. Samukawa, K.; Yamashita, H.; Matsuda, H.; Kubo, M. Simultaneous analysis of saponins in ginseng radix by high performance liquid chromatography.Chem. Pharm. Bull. 43,137-141 (1995).

201. Kwon, J. H.; Belanger, J. M. R.; Pare, J. R. J.; Yaylayan, V. A.Application of the microwaveassisted process

(MAP) to the fastextraction of ginseng saponins.Food Res. Int. 36, 491-498 (2003).

202. Fuzzati, N.; Gabetta, B.; Jayakar, K.; Pace, R.; Peterlongo, F.Liquid chromatography-electrospray mass spectrometric identification of ginsenosides inPanax ginsengroots.J. Chromatogr.A854,69-79 (1999).

203. Court, W. A.; Hendel, J. G.; Elmi, J. Reversed-phase highperformance liquid chromatographic determination of ginsenosides ofPanax quinquefolium.J. Chromatogr. A755,11-17 (1996).

204. Wu, J.; Lin, L.; Chau, F.-T. Ultrasound-assisted extraction ofginseng saponins from ginseng roots and cultured ginseng cells.Ultrason. Sonochem.8, 347-352 (2001).

205. Cui, J. F. Identification and quantification of ginsenosides invarious commercial ginseng preparations.Eur. J. Pharmacol.Sci.,3,77-85 (1995).

206. Wu, J.; Lin, L.; Chau, F.-T. Ultrasound-ssisted extraction ofginseng saponins from ginseng roots and cultured ginseng cells.Ultrason. Sonochem. 8, 347-352 (2001).

207. Choi, M. P. K.; Chan, K. K. C.; Leung, H. W.; Huie, C. W.Pressurized liquid extraction of active ingredients (ginsenosides)from medicinal plants using nonionic surfactant solutions.J.Chromatogr.A983, 153-162 (2003).

208. Ji, Q. C.; Harkey, M. R.; Henderson, G. L.; Gershwin, M. E.;Stern, J. S.; Hackman, R. M. Quantitative determination ofginsenosides by high-performance liquid chromatography-tandem mass spectrometry.Phytochem. Anal. 12, 320-326 (2001)

209. Fang, Q.; Yeung, H. W.; Leung, H. W.; Huie, C. W. Micelle-mediated extraction and preconcentration of

ginsenosides fromChinese herbal medicine.J. Chromatogr. A904,47-55 (2000).

210. Chan, T. W. D.; But, P. P. H.; Cheng, S. W.; Kwok, I. M. Y.;Lau, F. W.; Xu, H. X. Differentiation and authentication ofPanaxginseng,Panax quinquefolius, and ginseng products by usingHPLC/MS.Anal. Chem. 72, 1281-1287(2000)

211. Mizuno, M.; Yamada, J.; Terai, H.; Kozukue, N.; Lee, Y. S.;Tsuchida, H. Differences in immunomodulating effects betweenwild and culturedPanax ginseng.Biochem. Biophys. Res.Commun. 1672-1678 (1994,200)

212. Jhang, J. J.; Staba, E. J.; Kim, J. Y. American and Korean ginsengtissue cultures: growth, hemical analysis, and plantlet production.In Vitro,9, 253-259 (1974)

213. Kim, W. Y.; Kim, J. M.; Han, S. B.; Lee, S. K.; Kim, N. D.;Park, M. K.; Kim, C. K.; Park, J. H. Steaming of ginseng athigh temperature enhances biological activity.J. Nat. Prod. 63, 1702-1704 (2000)

국문초록

인삼사포닌(진세노사이드)은 인삼의 대표적인 활성성분으로 특히 항당뇨, 항염증 및 항암작용과 관련이 있다. 인삼의 잎이나 과실, 그리고 특히 뿌리 부분에 포함되어 있는 진세노사이드를 얻고 이를 정량하기 위하여 다양한 추출, 정제 및 분석방법이 사용되어 왔다. 추출법은 일반인삼과 가공인삼 으로부터 얻어진 진세노사이드의 전처리에 있어서 중요한 과정으로서, 보편적으로 사용되는 용매추출법에서는 적절한 용매의 사용, 가열 및 교반등을 통하여 용해도 혹은 용해속도를 증가시킨다.

본 연구에서는 일반백삼과 열처리한인삼의 최적추출조건을 결정하고 각 진세노사이드를 정량하였다. 특히, 추출용매의 극성이 추출효율에 미치는 영향을 집중적으로 연구하였다. 이때, 열에의한 변성을 최소화하기 위하여 비교적 낮은온도인 40℃에서 실험하였다.

*P.ginseng*으로부터얻어진백삼과열처리한인삼에함유되어있는아홉종류의진세노사이드(Rb1, Rb2, Rc, Rd, Re, Rg1, (20*S*)-Rg3, Rg5, Rk1)를 추출하기 위하여 다섯 종류의 메탄올-물 혼합액(50, 60, 70, 80, 90% 메탄올)이 사용되었으며 이 혼합

액들은 서로 다른 추출효율을 보였다.

최적조건은 백삼과 열처리한인삼 모두에 대하여 60% 메탄올을용매로 사용할 때인 것으로 나타났다. 다만, 진세노사이드 Rg5와 Rk1의 효율적인 추출을 위해서는 80% 메탄올이 가장 좋은 것으로 나타났다. 이로부터 진세노사이드 Rg5와 Rk1을 다량 함유하는 인삼가공품의 경우에는 상대적으로 극성이 낮은 용매를 사용하여 추출하는 것이 효율적일 것으로 예측 해 볼 수 있다.

한편, 아홉 종류의 진세노사이드의 양과 HPLC크로마토그램에서의 피크면적과의 상관계수의 제곱은 모두 0.994 이상이었으며, 검출한계는 1 에서 7 ng/mL 사이, 정량한계는 2 에서 23 ng/mL 사이인 것으로 나타났다. 본 연구에서 제안된 분석방법은 백삼과 열처리 한 인삼에 함유되어 있는 아홉종류의 진세노사이드에 성공적으로 적용될 수 있었다.

주요어: *Panax ginseng*, 열처리인삼, 진세노사이드, 추출, HPLC, 자외-가시부흡광검출기

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